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# Canadian Journal *of* Research

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VOLUME 11 SEPTEMBER, 1934 NUMBER 3

## ON THE THERMAL CONDUCTIVITY OF DIATOMITE AT MODERATELY HIGH TEMPERATURES[1]

BY C. D. NIVEN[2]

### Abstract

An examination was made of various kinds of diatomite brick, in order to ascertain how the thermal conductivity of diatomite found at Quesnel in British Columbia compared with the conductivities of some other diatomites. A brief description is given of the difficulties which attend any endeavor to measure thermal conductivity with accuracy above 1500° F. and which necessitate the limiting of the investigation to temperatures between atmospheric temperature and 1500° F. Of the samples investigated the Quesnel brick was found to be the best thermal insulator with the exception of a brick consisting of natural Californian diatomite.

### Introduction

As an insulator at high temperature there are few substances which can surpass diatomite. This material is found in deposits in various parts of the world, notably in California, in the Island of Moler off the north coast of Denmark, and in Canada. It "consists of minute fossil envelopes of diatoms, a family of unicellular plants," and after it is calcined, it is about 90% silica. The deposits often occur along with clay. When this is so, the clay is sometimes used to bind the diatomaceous earth together in the manufacture of insulating bricks. The insulating qualities of these bricks are of greater importance than their structural strength, as they are used for the inside of furnace walls—the outer faces of which can be made of fire clay brick on the one side and of ordinary brick on the other.

In central British Columbia valuable deposits of this diatomaceous material occur at Quesnel, and the Department of Mines in Canada have been anxious to ascertain whether the British Columbian product would compare favorably with other diatomites as a thermal insulator. On account of the fact that diatomite can be used for insulation at very high temperatures, it was considered desirable to carry out the investigation at the highest mean temperature possible; but when the matter was looked into, the difficulties of

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*Contribution from the Division of Physics and Engineering, National Research Laboratories, Ottawa, Canada.*

[2] *Physicist, National Research Laboratories, Ottawa.*

accurately measuring thermal conductivity at very high temperatures appeared to be very considerable if not entirely insurmountable. At any rate no standard method had been developed for such work.

Three principles are available for estimating the transmission of heat energy: first, the measurement of heat supplied; second, the measurement of the heat which comes through the insulator and is absorbed on the cold side; third, the measurement of heat flow across any section parallel to the hot or cold plate. Each of these methods has its disadvantages.

The first principle, which in point of fact was the one employed by the writer, cannot be used for hot plate temperatures above 1600° F. This results from the fact that no material which is a good electrical insulator at these temperatures has yet been found and therefore no material is available, upon which to wind the heater wire supplying the thermal energy, without involving an electrical leak whereby the accuracy of the results would be vitiated.

The second principle known as the calorimeter principle depends upon the flow of water at constant speed through the cold plates. The rise in temperature of the water gives a measure of the heat transmitted. This method, however, practically fixes the cold plate temperature at about 100° F. so that to reach a mean temperature of 1500° F. the hot plate temperature would have to be in the neighborhood of 2900° F. This principle, therefore, does not permit of any higher mean temperatures than the first one; it works satisfactorily and is used at the National Physical Laboratory in England.

The third principle is still in the experimental stages and need not be discussed here. If one can get a satisfactory flow meter, this method has the overwhelming advantage over the other two, that neither the temperature of the hot, nor the temperature of the cold plate, need be limited to narrow ranges since neither plate is used for the measuring of the energy transmitted.

## Apparatus

A design of apparatus was employed, very similar to that described by Hartmann, Westmont and Weinland (1). It will be necessary therefore to refer only to the main features of the design.

This type of apparatus differs from the ordinary hot plate apparatus in that the "cold" plates also are heated, and so comparatively high temperatures can be obtained on the cold side of the sample. The cold plate consists essentially of four parts, namely, a water-cooled plate, a piece of sil-o-cel insulation, an electrically heated alundum plate, and a nichrome disk—the last mentioned being employed to give equal distribution of heat over the cold face of the test sample.

The hot plate itself and the two heated plates used in the combinations which formed the cold sides were constructed from double-spiral-grooved alundum plates. The heater wire which rested in this double-spiral groove was a No. 22 nichrome wire. Nichrome is less expensive than platinum and must therefore be used, but great trouble was caused by the wire oxidizing

after long periods of heating at the high temperatures necessary for the work. The hot plate was equipped, of course, with a guard-ring, and platinum-platinum-rhodium thermocouples cemented into the surface of the plate determined when the guard-ring was at the same temperature as the central portion of the plate.

The energy supplied per square foot of plate surface was estimated by measuring the current flowing through the nichrome wire and the difference of potential at two points on the wire; these two points were situated roughly at the points at which the nichrome heater wire crossed the circumference of a circle of a radius of two inches, the centre of which was at the centre of the plate. The actual area of this circle was measured with a planimeter on a carbon rubbing, taken after the spiral had been wound and the potential leads welded on. The energy supplied per square foot of surface was thus the product of a voltage reading, an amperage reading and a constant, obtained once and for all for a particular plate. Mention might be made again of the necessity of using a good electrical insulator for the substance of which the heater plate is composed; the very method of winding, namely by means of a double spiral, implies that the electrical leak—which of course depends on the difference of potential—would vary over a plate which was slightly conducting. It would be greatest at the edge and smallest at the centre.

## Experimental Procedure

The materials for testing were provided in the form of bricks. These samples had to be cut to form a slab one inch thick and eight inches in diameter. In order that no join might come in the central four inches of the slab, the following method of cutting was adopted. The brick was cut in two, parallel to the face of the largest area; then one of the two halves so obtained was cut diagonally. These two triangular pieces were placed one on either side of the other, or uncut, half of the brick, and the eight-inch circular slab was cut out. The thickness of a slab thus prepared was measured in a micrometer gauge, and then two platinum-platinum-rhodium thermocouples were fastened, one on either side, with de Khotinsky wax. The couples were sunk into the faces of the sample, so that the junction was flush with the surface. Half of the sum of the thicknesses of the two couples was subtracted from the micrometer reading and the resulting value was taken as the thickness measurement. In placing the samples in the apparatus a thin sheet of asbestos cardboard was placed between the sample and the nichrome disk of the cold plate combination, in order to insulate electrically the thermocouples from that disk.

As a rule it took from two to four days to find the values corresponding to a steady flow of heat. The steady state was taken at mean temperatures in the neighborhood of 500°, 1000° and 1500° F. In this way, the variation of thermal conductivity with temperature was ascertained.

## Results and Conclusions

The results obtained are given in Table I. These are shown again in Fig. 1 where thermal conductivity has been plotted against temperature. The

TABLE I

| Description of sample | Reference number on Fig. 1 | Density (lb./cu. ft.) | Temperature (° F.) | Thermal conductivity (B.T.U./hr./sq. ft./° F./in.) | Remarks |
|---|---|---|---|---|---|
| Californian diatomite: clay bond; calcined | 1 (a) | 40.1 | 441 | 1.54 | |
| | | | 997 | 1.80 | |
| | | | 1378 | 1.91 | |
| Californian diatomite: clay bond; calcined | 1 (b) | 38.6 | 196 | 1.53 | |
| | | | 471 | 1.63 | |
| | | | 673 | 1.74 | |
| | | | 892 | 1.84 | |
| | | | 1193 | 1.98 | |
| | | | 1386 | 2.05 | |
| | | | 1557 | 2.14 | |
| Californian diatomite: cut out of the deposit; no bond; not calcined | 2 | 29.7 | 536 | 0.556 | |
| | | | 887 | 0.666 | This brick was not calcined and showed marked change in color at higher temperatures; a slight contraction was also noted |
| | | | 1229 | 0.677 | |
| | | | 1437 | 0.666 | |
| Californian diatomite: a brick supposed to be specially designed for high temperatures | 3 | 38.4 | 462 | 1.33 | This brick was supposed to stand very much higher temperatures than the maximum at which it was tested but it showed a slight change of color as a result of testing at 1412° F. |
| | | | 988 | 1.54 | |
| | | | 1217 | 1.57 | |
| | | | 1412 | 1.59 | |
| Moler Island diatomite: clay bond | 4 | 29.1 | 474 | 0.906 | Deep holes were visible on the surface of this sample; it is understood that something is put in, in the manufacture of this brick which is subsequently burnt out |
| | | | 1020 | 1.18 | |
| | | | 1406 | 1.41 | |
| Moler Island diatomite: clay bond | 5 | 49.3 | 432 | 1.31 | |
| | | | 1056 | 1.53 | |
| | | | 1517 | 1.72 | |
| (Uncertain origin; probably mixture of Californian and Algerian diatomite) | 6 | 32.0 | 440 | 1.04 | Samples 6 and 7 are probably a mixture of Californian and Algerian diatomite; before calcining it is understood they have a percentage of cork granules which are subsequently burnt out. No. 6 is a reddish colored brick. No. 7 is white |
| | | | 965 | 1.29 | |
| | | | 1437 | 1.59 | |
| (Uncertain origin; probably a mixture of Californian and Algerian diatomite with a different bond from No. 6) | 7 | 37.8 | 455 | 1.22 | |
| | | | 1080 | 1.63 | |
| | | | 1517 | 1.92 | |
| British Columbian diatomite from Quesnel: clay bond; calcined | 8 | 30.7 | 490 | 0.801 | This brick is rather easily broken and is softer than the other bricks |
| | | | 1092 | 1.02 | |
| | | | 1499 | 1.17 | |

relation between thermal conductivity and temperature for each sample appeared in general to be expressed by a straight line. The accuracy of the work did not justify the drawing of curves through points which did not strictly obey the linear relation. By extrapolation one may form an estimate of the insulating value of the materials at higher temperatures than those at which the experimental work was undertaken, but such a procedure implies that no change takes place in the physical state of the sample in question, in the region of temperature over which the extrapolation is made.

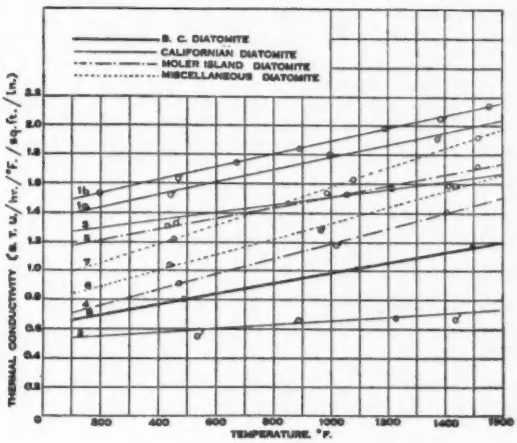


FIG. 1

The work definitely shows that in the range of temperature between 400 and 1500° F. the conductivity of the Quesnel sample is less than that of any of the other samples except the natural Californian diatomite brick which is cut out of the solid deposit and does not contain what is known in ceramics as a "bond."

## Acknowledgments

The author takes pleasure in acknowledging many courtesies shown by the Johns-Manville Laboratories, and in particular by Mr. C. E. Weinland, who gave much valuable advice in connection with the construction of the testing apparatus.

## Reference

1. HARTMANN, M. L., WESTMONT, O. B. and WEINLAND, C. E. Proc. Am. Soc. Testing Materials, Part II, 28 : 820-847. 1928.

# LONGITUDINAL WAVES IN CYLINDERS OF LIQUID, IN HOLLOW TUBES AND IN SOLID RODS[1]

By Geo. S. Field[2]


## Abstract

A consideration of recently published results on the transmission of longitudinal waves in cylinders of liquid, in hollow tubes and in solid rods shows that the first two cases give velocity-frequency curves which are quite similar to each other but different from that obtained with the third case. The reason for these results is discussed and is shown to be most probably the relative importance of shear forces in each case. The effect of viscosity on the phase velocity of longitudinal waves is considered, and it is shown that the classical method of introducing viscosity leads to erroneous results.


## Introduction

As the result of an investigation on the transmission of sound in liquids contained in cylindrical tubes, it was shown (5) that a discontinuity in the relation between velocity and frequency occurs when the frequency of the longitudinal vibration approaches that of the fundamental radial vibration. The plotted results have the appearance of Curve 1, Fig. 1.

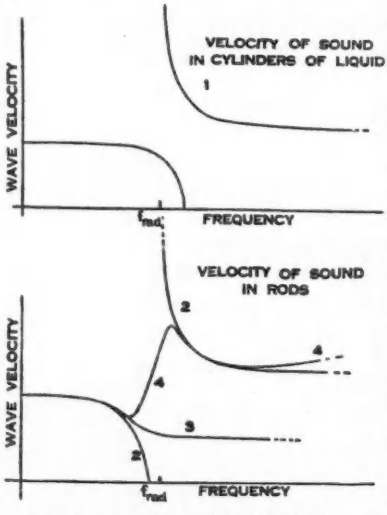


FIG. 1. *Variation of phase velocity with frequency for sound in liquids and in rods.* $f_{rad}$ *is the frequency of radial resonance.*

Recently a paper was published (6) on the longitudinal oscillations of solid rods and tubes, and a curve was given showing velocity plotted against frequency for a nickel tube. This curve resembles Curve 2, Fig. 1, and can be obtained theoretically, as the authors show, by a consideration of the simple theory of coupled oscillations. The results for solid rods, however, could not be explained so simply.

The vibrations in solid rods have been considered theoretically by Ruedy (12) and Field (4), and a paper containing some excellent experimental work was recently published by Röhrich (11). The variation of wave velocity with frequency is essentially as given by Curve 3, Fig. 1.

It seems of interest to determine why the occurrence of radial vibrations does not cause an abrupt change in the phase velocity of longitudinal waves in a solid rod, when it does do so in the other two cases. In what follows a reason for this behavior is advanced.

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*Contribution from the Division of Physics and Engineering, National Research Laboratories, Ottawa, Canada.*
[2] *Physicist, National Research Laboratories, Ottawa.*

## The Importance of Shear Forces

If, in the derivation of the velocity equation for solid rods, the forces due to shear be omitted, that is, it be assumed that the *net* shear strain is zero, the resulting equation is,

$$c_1 = \alpha\sqrt{1-\left(\frac{\sigma}{1-\sigma}\right)^2\frac{f_r^2}{f_r^2-f^2}} \quad *$$

where, $c_1$ = phase velocity;

$\alpha = c_o\sqrt{\frac{1-\sigma}{(1+\sigma)(1-2\sigma)}}$ = velocity of compressional wave in an infinite solid;

$c_o = \sqrt{E/\rho}$ = velocity of sound in a long, thin rod;

$E$ = Young's modulus; $\rho$ = density; $\sigma$ = Poisson's ratio;

$f$ = frequency; $f_r = \frac{x\alpha}{2\pi a}$; $a$ = radius of rod;

$x$ = solution of equation

$$x\frac{J_1(x)}{J_o(x)} = \left(\frac{1-\sigma}{1-2\sigma}\right)\frac{4\pi^2a^2f^2}{\alpha^2} \quad .$$

Upon plotting this equation, Curve 2, Fig. 1, is obtained, in which again the discontinuity in phase velocity appears, though it did not appear when shear forces were included. It seems reasonable to conclude, therefore, that it is these shear forces which tend to keep the phase velocity from becoming either very small or very large.

It is of interest to derive the velocity equation again†, this time with factors introduced to take care of viscosity. If such be done, Curve 4 is obtained. It will be observed that viscous forces, as well as shear forces, tend to eliminate high and low values of phase velocity, though the actual variation of velocity with frequency is different in the two cases.

Some idea of the way shear forces affect the longitudinal waves in a rod may be obtained if the changes in the shape of the rod as it vibrates are considered. Axial vibrations (on account of the lateral strain accompanying the longitudinal strain) produce radial waves, and hence cause periodic changes in the radius along the rod. Fig. 2 shows (very much exaggerated) how the rod responds to such waves travelling along it. It will be observed that shear waves are forced along the rod at the velocity of the longitudinal waves. If shear waves were oscillating freely along the rod, however, their velocity would be $c_o\sqrt{\frac{1}{2(1+\sigma)}}$, which, for duralumin, is a velocity only about 60% of that of a longitudinal wave in a long, thin rod. The longitudinal velocity, however, drops fairly rapidly as the frequency of the fundamental radial vibration is reached, until it approaches the velocity of shear waves as given above. At this value the shear waves accompanying

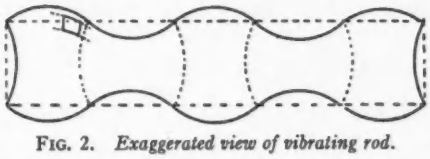

FIG. 2. *Exaggerated view of vibrating rod.*

* *See Equation (13), page 259.* † *See Equation (16), page 260.*

the longitudinal waves are in resonance with their natural frequency, and hence tend to maintain the velocity of the whole system of waves, *i.e.*, longitudinal and shear waves, at this value. Apparently this resonance effect is sufficient to prevent the velocity of the axial vibration from dropping much lower, and it continues near this value to higher and higher frequencies. (See Curve 3).

For liquids contained in tubes, of course, shear forces play no part. Hence, as the frequency is varied through the frequency of radial resonance, the phase velocity tends to become very low and then very high, limited only by viscosity.

In the case of thin tubes, also, shear forces are relatively unimportant. As radial vibrations occur, distortion of the walls into some such shape as Fig. 2 is accompanied by extension and bending, rather than by shearing. Therefore, in the absence of effective shear forces, a discontinuity in the velocity-frequency curve appears.

Reference to Equation (1) shows that at frequencies greater than that of the absorption band the velocity of compressional waves in an infinite solid is approached. Experiments (2, 3) have shown that the longitudinal velocity of sound in thin disks has nearly this value, so it seems reasonable to conclude that in such disks shear forces are relatively small.

## I. Derivation of Velocity Equation with Shear Forces Omitted

### Axial Strains in a Unit Cube

Elastic theory (1, p. 110, Equation (6) ), gives the following equations,

$$\begin{aligned} f_1(1+\sigma)(1-2\sigma) &= a(1-\sigma) + (b+c)\sigma \\ f_2(1+\sigma)(1-2\sigma) &= b(1-\sigma) + (c+a)\sigma \\ f_3(1+\sigma)(1-2\sigma) &= c(1-\sigma) + (a+b)\sigma \end{aligned} \qquad (1)$$

where $f_1$, $f_2$ and $f_3$ are the individual axial strains caused by the axial forces, $p_1$, $p_2$ and $p_3$, respectively, acting mutually at right angles to each other, and $a$, $b$ and $c$ are the *total* axial strains in the respective three directions. $\sigma$ is Poisson's ratio.

### Stresses and Strains in an Element of a Cylindrical Rod

Using the cylindrical co-ordinates, $r$, $\theta$ and $z$, consider an element with sides $rd\theta$, $dr$ and $dz$.

Let $z$ move to $z+\xi$. Then $z+dz$ moves to $z+dz+\xi+\frac{\partial \xi}{\partial z} \cdot dz$. The elongation of the element is $\frac{\partial \xi}{\partial z} \cdot dz$ and the strain is $\frac{\partial \xi}{\partial z}$.

Similarly, when $r$ moves to $r+\zeta$, the strain is $\frac{\partial \zeta}{\partial r}$, and when $\theta$ moves to $\theta+\eta$, the strain is $\frac{\partial \eta}{\partial \theta}$.

In this last case, however, there is an additional strain caused by the radial motion along $r$. An element $rd\theta$ wide is increased to a width $(r+\zeta)d\theta$ owing

to the radial motion through a distance $\zeta$. The strain thus caused is $\frac{\zeta}{r}$.

Hence the total strain in the direction determined by $rd\theta$ is, $\frac{\partial \eta}{\partial \theta} + \frac{\zeta}{r}$.

Finally, we can write,

Total strain along $z = \frac{\partial \xi}{\partial z} = a$,

Total strain along $r = \frac{\partial \zeta}{\partial r} = b$,

Total strain along $rd\theta = \frac{\partial \eta}{\partial \theta} + \frac{\zeta}{r} = c$. (2)

If Young's modulus be denoted by $q$, $p_1 = qf_1$, $p_2 = qf_2$ and $p_3 = qf_3$.

Substituting for the $f$'s from Equations (1), and using $a$, $b$ and $c$ as given by Equations (2), the $p$'s may be expressed as follows:

$$p_1 = \frac{q}{(1+\sigma)(1-2\sigma)}\left[\frac{\partial \xi}{\partial z}(1-\sigma) + \sigma\left(\frac{\partial \zeta}{\partial r} + \frac{\partial \eta}{\partial \theta} + \frac{\zeta}{r}\right)\right]$$

$$p_2 = \frac{q}{(1+\sigma)(1-2\sigma)}\left[\frac{\partial \zeta}{\partial r}(1-\sigma) + \sigma\left(\frac{\partial \xi}{\partial z} + \frac{\partial \eta}{\partial \theta} + \frac{\zeta}{r}\right)\right]$$

$$p_3 = \frac{q}{(1+\sigma)(1-2\sigma)}\left[\left(\frac{\partial \eta}{\partial \theta} + \frac{\zeta}{r}\right)(1-\sigma) + \sigma\left(\frac{\partial \xi}{\partial z} + \frac{\partial \zeta}{\partial r}\right)\right] \quad (3)$$

## Dynamical Equations

Remembering that the $p$'s are forces per unit area, the total force at $z$ in the $z$-direction is, $p_1 r d\theta \,.\, dr$.

Similarly, the total force at $z+dz$ is, $p_1 r d\theta \,.\, dr \,. + \frac{\partial}{\partial z}(p_1 r d\theta \,.\, dr)dz$.

Hence, the difference in tension on the two faces of the element causing acceleration in the $z$-direction is, $\frac{\partial}{\partial z}(p_1 r d\theta\, dr)\, dz$.

The net forces radially and tangentially are, likewise,

$\frac{\partial}{\partial r}(p_2 r d\theta \,.\, dz)dr - p_3 d\theta \,.\, dr \,.\, dz$ and $\frac{\partial}{\partial \theta}(p_3 dr \,.\, dz)d\theta$, respectively.

If the above forces be equated to the resulting changes in momentum, we have, after crossing out differentials on both sides of the equations,

$$\frac{\partial p_1}{\partial z} = \rho \frac{\partial^2 \xi}{\partial t^2} \quad (4)$$

$$r\frac{\partial p_2}{\partial r} + p_2 - p_3 = \rho r \frac{\partial^2 \zeta}{\partial t^2} \quad (5)$$

$$\frac{\partial p_3}{\partial \theta} = \rho \frac{\partial^2 \eta}{\partial t^2}$$

where $\rho$ is the density of the material.

If purely longitudinal and radial vibrations be considered, $\eta = 0$, $\frac{\partial^2 \eta}{\partial t^2} = 0$, and we are left with Equations (4) and (5). In them substitute for $p_1$ and $p_2$.

$$\alpha^2 \frac{\partial^2 \xi}{\partial z^2} + \beta^2\left(\frac{\partial^2 \zeta}{\partial r \,.\, \partial z} + \frac{1}{r}\frac{\partial \zeta}{\partial z}\right) = \frac{\partial^2 \xi}{\partial t^2} \quad (6)$$

$$\alpha^2\left(\frac{\partial^2 \zeta}{\partial r^2} + \frac{1}{r}\frac{\partial \zeta}{\partial r} - \frac{\zeta}{r^2}\right) + \beta^2 \frac{\partial^2 \xi}{\partial r \,.\, \partial z} = \frac{\partial^2 \zeta}{\partial t^2} \quad (7)$$

where,

$$\alpha^2 = \frac{q(1-\sigma)}{\rho(1+\sigma)(1-2\sigma)}$$

$$\beta^2 = \frac{q\sigma}{\rho(1+\sigma)(1-2\sigma)} \ .$$

If shear forces had been included, the left-hand side of Equation (6) would contain the term,

$$\gamma^2\left(\frac{\partial^2\xi}{\partial r^2} + \frac{1}{r}\frac{\partial\xi}{\partial r} + \frac{\partial^2\zeta}{\partial r \, . \, \partial z} + \frac{1}{r}\frac{\partial\zeta}{\partial z}\right)$$

and the left-hand side of Equation (7) would contain,

$$\gamma^2\left(\frac{\partial^2\zeta}{\partial z^2} + \frac{\partial^2\xi}{\partial r \, . \, \partial z}\right)$$

where, $\gamma^2 = \dfrac{q}{2\rho(1+\sigma)}$ = (velocity of shear waves in a solid)$^2$.

When the above shear terms are included in Equations (6) and (7), as well as the terms in $\eta$, these equations agree with those given by Love (8, p. 291).

## Solution of Dynamical Equations

Assume as solutions,

$$\xi = A f_1(r) \, e^{i\omega(t - z/c_1)}$$

$$\zeta = B f_2(r) \, e^{i\omega(t - z/c_1)}$$

where, $c_1$ = phase velocity, $\omega = 2\pi \times$ frequency; $t$ = time; and $f_1(r)$ and $f_2(r)$ are functions only of $r$, and $A$ and $B$ are constants.

Substitute the assumed solutions in Equation (6) and differentiate with respect to $r$. Then multiply by $-\dfrac{\beta^2}{\alpha^2}$.

Substitute the solutions in Equation (7) and multiply by $\dfrac{\alpha^2 - c_1^2}{\beta^2}$.

The two equations resulting from the foregoing may now be added together to give,

$$r^2\frac{\partial^2 f_2}{\partial r^2} + r\frac{\partial f_2}{\partial r} + (k^2r^2 - 1)f_2 = 0 \tag{8}$$

where,

$$k^2 = \frac{\alpha^2(\alpha^2 - c_1^2)}{\alpha^2(\alpha^2 - c_1^2) - \beta^4} \cdot \frac{\omega^2}{\alpha^2} \ . \tag{9}$$

Equation (9) may be re-written as,

$$c_1^2 = \alpha^2\left(1 - \frac{\beta^4}{\alpha^4} \cdot \frac{k^2\alpha^2}{k^2\alpha^2 - \omega^2}\right) . \tag{10}$$

The solution of (8) is, $f_2 = J_1(kr)$, so that,

$$\zeta = B J_1(kr) \, e^{i\omega(t - z/c_1)}.$$

Substituting this value in either of Equations (6) or (7) leads to,

$$\xi = \frac{Bc_1}{i\omega k\beta^2}(k^2\alpha^2 - \omega^2)\partial J_0(kr) e^{i\omega(t - z/c_1)}.$$

## Boundary Conditions

At $r = a$, $p_2 = 0$.

Substituting the expression for $p_2$ leads to,

$$(1-\sigma)\frac{\partial\zeta}{\partial a} + \sigma\left(\frac{\partial\xi}{\partial z} + \frac{\xi}{a}\right) = 0 \ .$$

If now the values for $\xi$ and $\zeta$ be used in this equation, and $x$ be written for $ka$, we have, finally,

$$\frac{J_1(x)}{xJ_o(x)} = \left(\frac{1-\sigma}{1-2\sigma}\right)\frac{4\pi^2\alpha^2f^2}{\alpha^2} \quad . \tag{11}$$

## Frequencies of Radial Resonance

Examination of Equation (10) reveals that $\frac{k\alpha}{2\pi}$ has the dimensions of frequency, and that $c_1$ becomes infinite when $\omega = k\alpha$; that is, anomalous dispersion occurs at this point. By analogy with the results obtained for liquids (5), it may be concluded that $\frac{k\alpha}{2\pi}$ represents the frequencies of resonant radial vibrations in the solid rod.

To determine the values of $k$ appropriate to the conditions, derive Equation (11) again, with $\frac{\partial\eta}{\partial\theta} = \frac{\partial\xi}{\partial z} = 0$; that is, in using the boundary condition, consider pure radial vibrations. Instead of Equation (11), the following equation then results,

$$\frac{(1-\sigma)}{(1-2\sigma)}xJ_o(x) = J_1(x) \tag{12}$$

where again $ka = x$.

The frequencies determined by the expression, $\frac{k\alpha}{2\pi}$, agree with those given by Ruedy (12).

## Velocity Equation

From Equation (10), if $f_r$ be written for $\frac{k\alpha}{2\pi}$, we have,

$$c_1^2 = \alpha^2\left[1 - \left(\frac{\sigma}{1-\sigma}\right)^2\frac{f_r^2}{f_r^2 - f^2}\right] \tag{13}$$

since $\omega = 2\pi f$, where $f$ is the frequency, and $\frac{\beta^4}{\alpha^4} = \frac{\sigma^2}{(1-\sigma)^2}$ .

## II. Derivation of Velocity Equation with Viscosity Included

In order to include viscosity in the analysis, we shall follow the procedure developed by Stokes (13) and consider the viscous forces as being proportional to the time rate of shearing strain ($s$), that is, equal to $\frac{\mu ds}{dt}$ , where $\mu$ is the coefficient of viscosity.

In Section I it was assumed that no net shearing strains existed, and it might appear, therefore, that the effect of viscosity would then be zero. However, it may be shown (1, p. 110) that any strain (a simple elongation, for example) may be considered as being compounded of two simple shears and a dilatation. Hence in the following analysis, although the net strains ($a$, $b$, $c$), as in Section I, are purely axial, we shall consider them as being made up of the shears, ($e_1$, $-e_1$, 0) and ($e_2$, 0, $-e_2$), and the dilatation ($d$, $d$, $d$).

We have (1, p. 110, Equation (4) ), $d=\frac{1}{3}(a+b+c)$, $e_1=\frac{1}{3}(a-2b+c)$ and $e_2=\frac{1}{3}(a+b-2c)$.

If $k$ = volume elasticity and $n$ = modulus of rigidity,

$$\left.\begin{aligned} p_1 &= 3dk + 2n(e_1+e_2) \\ p_2 &= 3dk - 2ne_1 \\ p_3 &= 3dk - 2ne_2 \end{aligned}\right\} \tag{14}$$

On Stokes' assumption, if the coefficient $n$ be replaced by the operator $\left(n+\mu\frac{\partial}{\partial t}\right)$, the contributions of the viscous forces will be fully taken into account (9, p. 560). Remembering that,

$$k = \frac{q}{3(1-2\sigma)}, \qquad n = \frac{q}{2(1+\sigma)},$$

we have,

$$\left.\begin{aligned} p_1 &= \frac{q}{(1+\sigma)(1-2\sigma)}\left[(1-\sigma)a+(b+c)\sigma\right] + \frac{2}{3}\mu\frac{\partial}{\partial t}\left(2a-b-c\right) \\ p_2 &= \frac{q}{(1+\sigma)(1-2\sigma)}\left[(1-\sigma)b+(c+a)\sigma\right] + \frac{2}{3}\mu\frac{\partial}{\partial t}\left(a-2b+c\right) \\ p_3 &= \frac{q}{(1+\sigma)(1-2\sigma)}\left[(1-\sigma)c+(a+b)\sigma\right] + \frac{2}{3}\mu\frac{\partial}{\partial t}\left(a+b-2c\right) \end{aligned}\right\} \tag{15}$$

In Equations (15), formed after recombination of the two shears and the dilatation, it will be noticed that the modulus of rigidity, $n$, does not appear, as it would if the *net* strains involved shearing. The moduli that do appear are the "elongational" elasticity, $\frac{q(1-\sigma)}{(1+\sigma)(1-2\sigma)}$, $\frac{q\sigma}{(1+\sigma)(1-2\sigma)}$, and the coefficient of viscosity, $\mu$. The forces due to the resolved shears and dilatation add together so that their resultant is the same as was obtained in Section I by dealing immediately with the net (purely axial) strains. The forces due to viscosity, however, cannot be combined with any force occurring as the result of dilatation. They therefore appear in each equation of (15) as an extra term. It is the same as though a further shearing force had been added, except that the force due to viscosity is 90° out of phase $\left(\text{on account of the operator } \frac{\partial}{\partial t}\right)$ with a shear force of equal numerical value.

Using the equations for $p_1$, $p_2$ and $p_3$, we proceed exactly as in Section I and find that now,

$$c_1^2 = \frac{\left[\alpha^2(\omega^2-k^2\alpha^2)+k^2\beta^4+\frac{3}{4}k^2\varphi^2\right]+\omega^2\varphi}{(\omega^2-k^2\alpha^2)+k^2\varphi}, \tag{16}$$

where

$$\varphi = \frac{4}{3}\frac{i\omega\mu}{\rho},$$

and that $k$ is given by the equation,

$$ka\frac{J_1(ka)}{J_0(ka)} = \left(\frac{1-\sigma}{1-2\sigma}\right)\frac{4\pi^2a^2f^2}{1-\frac{3\varphi}{2\alpha^2}\left(\frac{1-\sigma}{1-2\sigma}\right)} \cdot \frac{1}{\alpha^2}. \tag{17}$$

Equation (16) shows that $c_1$ is now complex. If $\frac{1}{c_1}$ be denoted by $\frac{1}{c_r} - i\delta$, the real phase velocity will then be $c_r$, and we shall have the damping term, $e^{-\delta\omega z}$, added to the expressions for the particle velocities.

When the equation for $c_r$ is plotted, Curve 4 results. The surprising thing that will be observed is that the phase velocity is *increased* at high frequencies. One would expect, of course, that viscosity would have the opposite effect. This peculiar result is discussed further in Section III.

## III. On the Solution of the Wave Equation with a Viscosity Term Included

In considering the effect of viscosity, Stokes (13) has been followed by Rayleigh (10, p. 315), Lamb (7, p. 186-188) and others in including viscosity as proportional to the time rate of shearing stress. To investigate the effect of such an assumption, it is convenient for a moment to consider simpler equations than Equation (6) or Equation (7) in Section I, and for this purpose we can take the one applying to rods when radial motion is neglected, and include in it a term to take care of viscosity. This equation then is (9, p. 561, Equation (7) ),

$$\frac{\partial^2 \xi}{\partial t^2} = c_0^2 \frac{\partial^2 \xi}{\partial x^2} + R \frac{\partial^3 \xi}{\partial x^2 \partial t} \tag{18}$$

where

$$R = \tfrac{4}{3} \frac{(1+\sigma)\mu}{\rho} .$$

Equation (18) is practically identical with that obtained for liquids (7, p. 188, Equation (12) ).

As a solution of Equation (18), we follow Rayleigh and Lamb, and assume,

$$\xi_1 = A e^{int + mx} . \tag{19}$$

From (18),

$$-n^2 = m^2 c_0^2 + Rm^2 in$$

or,

$$m^2 = \frac{-n^2}{c_0^2 + Rin} . \tag{20}$$

Let $m = a + ib$, and let the right-hand side of Equation (20) be denoted by $iA - B$.

Then, $a^2 - b^2 = -B$, and, $ab = \frac{A}{2}$ .

Hence

$$b^2 = \frac{B}{2}\left(1 + \sqrt{1 + \frac{A^2}{B^2}}\right) .$$

The phase velocity, $c_1$, is given by $\frac{n}{b}$, and therefore,

$$\frac{c_1^2}{c_0^2} = \frac{2(1+\theta^2)}{1+\sqrt{1+\theta^2}} \tag{21}$$

where,

$$\theta = \frac{Rn}{c_0^2} .$$

On account of the viscosity term, $\theta$, Equation (21) shows that the phase velocity, $c_1$, in a viscous medium will always be *greater* than the phase velocity, $c_o$, in a frictionless medium. This result can hardly be justified on physical grounds, and might indicate that viscosity has not been introduced correctly into the wave equation, or else that the assumed particular solution is not the correct one to use.

Instead of Equation (19) we may take,

$$\xi_2 = Ae^{mt+ikx} . \tag{22}$$

Then, $m^2 = -c_o^2k^2 - Rmk^2$.

And, if $m = a+ib$, we have,

$$b = kc_o\sqrt{1-\frac{R^2k^2}{4c_o^2}} . \tag{23}$$

Since the phase velocity is given by $\frac{b}{k}$, we have,

$$\frac{c_1}{c_o} = \sqrt{1-\frac{R^2k^2}{4c_o^2}} . \tag{24}$$

This expression for $c_1$ seems more plausible than the preceding one, as it indicates a lower value for the phase velocity than that for the velocity in a frictionless medium. An examination of the two assumed solutions of the wave equation, however, shows that the second solution is physically not very likely. The essential difference between the expression for $\xi_1$ and the expression for $\xi_2$ is that in the first case damping is assumed with space but not with time, and in the second case damping occurs with time but not with space. For $\xi_1$ we have waves going out from the origin with ever-decreasing amplitude as the distance $x$ increases, but at any time the amplitude of the waves at a point in space is the same as the amplitude at the same point at any other time. In the case of $\xi_2$ the waves from zero to infinity have all the same amplitude, and as time progresses the amplitude of each wave dies away at the same rate.

This latter conception is somewhat artificial and is certainly not true for waves progressing through a viscous medium. It is interesting, however, that it does give a more likely value for the phase velocity than the previous solution. Finally it may be remarked that it appears strange that the phase velocities should be different in the two cases, though it occurs probably on account of the physical unreality of the second assumed solution.

If, instead of taking viscous damping as proportional to the time rate of shearing, it be taken simply as proportional to the particle velocity, the term $R\frac{\partial^3\xi}{\partial x^2\partial t}$ in the wave equation, (Equation 18) will be replaced by $R\frac{\partial\xi}{\partial t}$. Then both the assumed solutions mentioned above result in values for the phase velocity which are lower than the velocity in a frictionless medium. This result alone, however, is hardly sufficient to justify the simpler method of introducing viscosity. But, in any case, there is something wrong with the classical method, and further investigation of this matter seems to be required.

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# RAPID DETERMINATION OF MOISTURE IN GRAIN

## I. COMPARISON OF 130°C. AIR OVEN AND BROWN-DUVEL METHODS WITH VACUUM OVEN METHOD[1]

By W. H. Cook[2], J. W. Hopkins[3] and W. F. Geddes[4]


### Abstract

Some 300 samples of hard red spring wheat, 50 of amber durum wheat and 75 each of oats and barley, covering the entire moisture range of commercial importance, were employed in the comparisons. Provision was made for statistical estimation of sampling errors, errors of method and errors of observation. A two-stage drying procedure was employed for the vacuum oven determinations which were taken as the reference standard. Sampling errors, average difference between identical ovens and errors due to daily fluctuations were all larger than the standard deviation of duplicates ("experimental error"), the sampling error being the largest.

A comparison of the Hobart and Wiley grinders shows the latter to be superior for preparing wheat for the 130° C. air oven determination. The air oven consistently underestimates the moisture content of all the grains tested, as compared with the vacuum oven, the deficit increasing with the actual moisture content. This systematic bias may be eliminated by means of a linear correction equation. After allowing for the observed sampling errors and errors of the vacuum oven determination, the net standard error of prediction with the air oven using a Wiley mill is, with hard red spring wheat 0.24%, with amber durum wheat 0.12%, with barley 0.20% and with oats 0.20% moisture.

The Brown-Duvel method also underestimates the moisture content, this deficit also increasing with the actual moisture content in the case of spring and amber durum wheat. After applying a correction equation to eliminate the systematic bias, the net standard error of prediction of moisture by this method was found to be: with hard red spring wheat 0.16%, with durum wheat 0.09%, with barley 0.12% and with oats 0.13%. The Brown-Duvel method, therefore, when carefully operated makes possible a more consistent estimate of the actual moisture content, as determined by the vacuum oven, than does the 130° C. air oven method. As with the vacuum oven, both the 130° C. air oven and the Brown-Duvel appear to be subject to slight variations affecting all the determinations made on any particular day.


## I. Introduction

Information respecting the amount of moisture contained in cereals is exceedingly important commercially. As the more accurate laboratory methods for estimating moisture content are ordinarily too slow and cumbersome, many rapid but somewhat empirical methods have been devised to meet the requirements of the grain trade, electrical moisture meters constituting the latest development in this connection. The object of the present investigation was a critical study of the utility and accuracy of a selected number of these rapid methods, including five electrical moisture meters of different make or design. Rapid analytical methods investigated were the Brown-Duvel and the 130° C. air oven. Other rapid analytical methods such as the carbide method for flour described by Blish and Hites (3) and the methyl alcohol method of Cashmore (5) were not tested as they are not in common

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[2] *Biologist, National Research Laboratories, Ottawa.*
[3] *Statistician, National Research Laboratories, Ottawa.*
[4] *Chemist-in-Charge, Dominion Grain Research Laboratory, Board of Grain Commissioners, Winnipeg.*

use in the trade. The electrical testers studied consisted of the Burton-Pitt, Davies, Limbrick, hand-operated Tag-Heppenstall and motor-operated Tag-Heppenstall instruments; the results obtained with these meters will be discussed in the next paper of this series.

The procedure in comparing the general reliability of these methods consisted of determining the moisture content of a subsection of some 300 samples of hard red spring wheat, 50 of amber durum wheat and 75 samples each of oats and barley, by each method simultaneously, with provision for statistical estimation of sampling errors, errors of method and ordinary experimental error. Actually the statistical computations were made on a somewhat smaller number of samples in each case, for in an investigation of this size there were a few inevitable losses due to accidents, etc. It was planned originally to include a similar number of rye and flax samples, but these grains proved not to be available in sufficient quantity for a statistical study. Samples of each grain were selected so that about one third of the total number fell into each of the moisture classes, "straight," "tough" and "damp," the exact moisture limits of which are shown in Table I. The grades encountered and the number of samples in each grade were determined by the quality of the grain received at the inspection points during the collection period. In general, and especially with spring wheat, the higher grades were more abundant than the lower ones, but the samples obtained cover the entire range of moisture content and grade normally found in commercial practice.

TABLE I

MOISTURE LIMITS OF STRAIGHT, TOUGH AND DAMP GRAIN*

| Kind of grain | Percentage of moisture (by official Brown-Duvel method) allowed | | |
|---|---|---|---|
| | Straight | Tough | Damp |
| Hard red spring wheat | Up to 14.5 | Over 14.5 but not over 17.0 | Over 17.0 |
| Durum wheat | Up to 14.8 | Over 14.8 but not over 17.0 | Over 17.0 |
| Oats | Up to 14.0 | Over 14.0 but not over 17.0 | Over 17.0 |
| Barley | Up to 14.8 | Over 14.8 but not over 17.0 | Over 17.0 |
| Rye | Up to 14.0 | Over 14.0 but not over 17.0 | Over 17.0 |
| Flax | Up to 10.5 | Over 10.5 but not over 13.5 | Over 13.5 |

**As established by the Board of Grain Commissioners for Canada.*

The vacuum oven was employed as a standard of comparison and two separate subsamples were tested by this method in order to obtain an estimate of the sampling error arising from the subdivision of the samples. In addition, any day-to-day variations in the results by the methods under investigation were studied by subdividing three large samples, one in each of the straight, tough and damp classes, into a large number of subsamples, and daily determining the moisture content of two of these by the vacuum oven method, and that of one of each daily by each of the other methods.

## 2. Experimental Material

### Collection and Storage of Samples

The grain samples used were obtained at various points in western Canada by the staff of the Western Grain Inspection Division. One-gallon samples were collected, placed in moisture-tight cans and shipped to the Dominion Grain Research Laboratory, Winnipeg, where they were cleaned and their approximate moisture content determined. A larger number of samples than required was collected in order to permit selection of the requisite number within each moisture class. Only some 30 samples of naturally damp wheat were received so that over 70 samples had to be artificially dampened by adding water in order to obtain the necessary damp samples. After being dampened with distilled water, the samples thus treated were allowed to stand for one month in moisture-tight cans in a room maintained at a temperature near the freezing point. The samples were removed from the cans every few days, thoroughly mixed and replaced in the cans. The naturally straight, tough, and damp samples were also stored under the above conditions for at least a month, with occasional mixing, before they were subdivided for the various tests. All of the damp samples of oats and barley tested were "naturally" damp when received, but as no naturally damp samples of amber durum wheat were available, certain straight grade and tough samples were moistened by exposure to high humidity for several days, dried back to the required moisture in the laboratory and stored before testing.

### Subdivision of Samples

It was necessary to divide each sample into eight subsamples and for this purpose the Boerner (4) sampler, recommended by the American Association of Cereal Chemists (1), would have been extremely slow and laborious. To have superimposed several such samplers one on another would have been unduly expensive. A one-to-eight progressive riffle was therefore designed and constructed. The principle of this apparatus is evident from Fig. 1. The chutes leading from one set of riffles to the next were placed at an angle of 32° to the horizontal. At this angle even excessively damp wheat would flow without stopping and in all cases the kernels spread quite uniformly across the chutes, thus insuring that the receiving riffle was fed uniformly over its entire width.

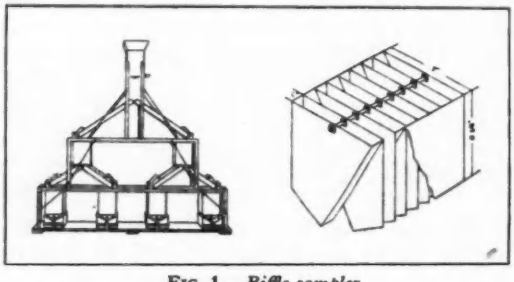

FIG. 1. *Riffle sampler.*

The individual riffle units consisted of eight sections one inch wide, adjoining sections discharging in opposite directions. An adjustment, for setting

each section in an individual riffle unit to receive the same quantity of grain, was provided by passing a threaded rod, with nuts on each side of each section, through the middle of the section in each riffle unit one inch below the top. By means of these nuts the sides of the sections could be adjusted so that each one discharged the same weight of grain. This adjustment was made on each section of each riffle before the sampler was assembled.

As the samples to be used had been kept for a month or more in moisture-tight cans, with periodic mixing, it was assumed that each sample was reasonably homogeneous with respect to moisture. The subdivision of these relatively small samples into eight subsamples appeared therefore to present a relatively simple sampling problem and the apparatus described above, after adjustment to give eight subsamples of practically equal size, was considered adequate for the purpose. Nevertheless, in order to obviate any possible systematic error, the eight spouts of the sampler were numbered and the corresponding subsamples distributed at random among the methods and instruments under test.

In practice the amount of grain sampled was only slightly in excess of that required to fill the eight half-pint double-tight paint cans employed as subsample containers. The sampling was done in a room in which the temperature was near the freezing point, and it was found that exposing even a damp sample for six hours under these conditions did not reduce the moisture content by any amount measurable by the Brown-Duvel method. The samples were divided two or three days before actual use and the resulting subsamples were then allowed to attain temperature equilibrium before testing by letting them stand for 24 hr. in the room in which the determinations were made.

## 3. Experimental Methods

### Preliminary Study of Technique

*Preparation of samples for vacuum oven*

Samples of whole grain must be ground before their moisture content can be determined by the oven (indirect) methods. Obviously the moisture loss during grinding will depend on the fineness of grinding, the type of grinder employed, the atmospheric conditions in the room in which the grinding is done, and the initial moisture content of the grain. Flohil and his collaborators (8) have already stressed the importance of these points.

The official method of the Association of Official Agricultural Chemists (9) demands that the sample should be ground to pass through 1-mm. circular openings. This procedure is qualified by the statement that if the sample cannot be thus ground it should be reduced to as fine a condition as possible. The method of the American Association of Cereal Chemists (1) requires 30–40 gm. of wheat to be ground to a floury consistency, or to such a degree of fineness that at least 50% of it will pass through a 36 grits gauze sieve. Neither of these descriptions specify the type of mill to be employed for grinding.

Two general types of grinders are available for reducing the size of the material. The attrition type, such as a Hobart mill, is capable of reducing the material, unless of high moisture content, to a fine floury consistency, but it has the disadvantage of heating the sample and thus increasing the moisture loss. The shearing type, such as the Wiley mill, does not heat the sample to nearly the same extent, but it gives a coarser granulation and requires a longer period for grinding. This involves longer exposure to the atmosphere of the grinding room with a resultant differential loss of moisture from samples of different initial moisture. The Brabender electro-magnetic grinder was tested in preliminary experiments, but when it was set for the finest granulation possible the material produced was still quite coarse. Moreover, under these conditions the mill heated appreciably and was impracticably slow.

In order to reduce the moisture loss due to exposure to the atmosphere and any slight loss that might occur from heating in the grinder, a two-stage drying method was employed. The first stage consisted of drying 100.00 ± 0.01 gm. of the wheat to equilibrium with an atmosphere of 40% relative humidity at 72° F. This was done in shallow dishes four by five inches in size. To accelerate the rate of drying during this initial stage, and also to avoid contamination with dust, the samples were conditioned in a ventilated cabinet which consisted essentially of a fan for drawing air over the samples, a series of shelves, a set of adjustable dampers for regulating the air flow, and a screen for excluding dust from the incoming air. A diagrammatic sketch of this cabinet is shown in Fig. 2. No heater or regulators were required as the atmospheric conditions in the room were controlled.

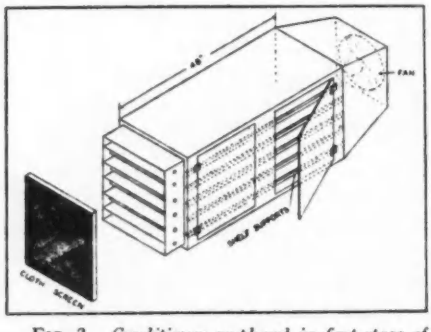


FIG. 2. *Conditioner employed in first stage of drying for vacuum oven determination.*

Under these conditions it was found that even the dampest samples attained approximate equilibrium in 72 hr., all of them then containing about 10.8% moisture. The samples were then re-weighed and ground in the same room, and 30 to 40 gm. of the ground material taken for the vacuum oven determination. The loss in weight during both stages was then computed in terms of moisture and expressed as a percentage of the weight of the original wet sample.

A preliminary experiment was made to compare the results obtained by the vacuum oven method using both the single and two-stage methods with a series of wheat samples of different initial moisture content. One portion of each sample was ground in a Hobart and one in a Wiley mill to compare the two methods of grinding. The Hobart mill was set to grind the material as finely as possible without choking, and a 1-mm. sieve was employed in the Wiley mill, since this meets the requirement of the official vacuum oven method (9). The results are given in Table II.

TABLE II

PERCENTAGE MOISTURE INDICATED BY VACUUM AND AIR OVENS USING DIFFERENT GRINDERS AND METHODS OF DRYING

| Sample No. | Vacuum oven, 5½ hr. | | Vacuum oven, 16 hr. | | 130° C. Air oven, 1 hr. | |
|---|---|---|---|---|---|---|
| | Wiley mill | Hobart grinder | Wiley mill | Hobart grinder | Wiley mill | Hobart grinder |
| Wheat ground directly (single-stage method) | | | | | | |
| 1 | 11.22 | 10.28 | 11.45 | 10.38 | 11.18* | 11.66 |
| 2 | 13.42 | 13.46 | 13.65 | 13.60 | 13.45 | 13.86 |
| 4 | 16.15 | 16.30* | 16.48 | 16.51* | 16.43 | 16.52 |
| 5 | 18.37 | 18.13 | 18.76 | 18.30 | 18.64 | 18.70 |
| Wheat dried to equilibrium with atmosphere before grinding (two-stage method) | | | | | | |
| 1 | 11.40 | 11.56 | 11.88 | 11.68 | 11.70 | 11.86 |
| 2 | 13.64 | 13.69 | 14.02 | 13.96 | 13.76 | 13.96 |
| 4 | 16.48 | 16.16 | 16.77 | 16.31 | 16.72 | 16.46 |
| 5 | 19.02 | 18.68 | 19.42 | 18.72 | 19.19 | 18.90 |

**Single determination only; duplicate lost.*
*All other figures are the average of duplicate determinations.*

In practically all cases, and especially with samples of high moisture content, the two-stage method shows higher moisture content than the single-stage method in which the wheat is ground directly. It seems unlikely that the additional respiration during the preliminary drying could account for a difference of this magnitude, and it is concluded that there is a moisture loss during grinding which justifies the use of the two-stage method for the vacuum oven determinations that are to be used as a standard of comparison. On the whole, samples ground in the Wiley mill show higher moisture content than those ground in the Hobart grinder, indicating that the heating which occurs in the latter causes a greater loss of moisture than that occasioned by the longer exposure necessary for grinding in the Wiley mill.

*Time of heating in vacuum oven*

Comparison of the moisture contents, given in Table II, after 5½ and 16 hours' heating in the vacuum oven shows quite definitely that the shorter period does not reduce the samples to constant weight. The vacuum oven was operated throughout in accordance with the official methods (1, 9) which require heating to constant weight, qualified by the statement "approximately 5 hours." Bailey (2) also points out, as the result of a collaborative study, that in many cases constant weight is not attained by five hours' drying. The period of heating required to give constant weight was therefore subjected to further test. Five samples of different initial moisture content were studied, using the single-stage method, each determination being made on triplicate portions of approximately 2.0 gm. These were removed from the vacuum oven at intervals varying from 5 to 20 hr. Twenty minutes was allowed for releasing the vacuum and the dishes were allowed to cool for 20

min. over calcium carbide before weighing. On replacing the dishes in the oven, 15 min. was allowed for evacuation and restoration of temperature equilibrium. The average moisture content obtained after each of the intervals is plotted in Fig. 3 (solid line).

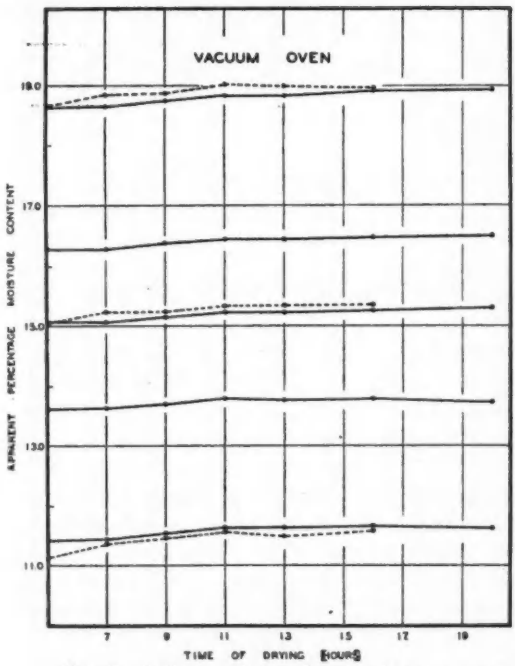


FIG. 3. *Influence of time of drying in vacuum oven on apparent moisture content of hard red spring wheat.*

Another experiment of this type was made using a slightly different procedure. Three samples were employed and 18 portions of approximately 2.0 gm. weighed out from each. These were all placed in the oven at the same time and three replicates from each sample removed at 5-, 7-, 9-, 11-, 13- and 16-hr. intervals respectively, cooled for 20 min. over $CaC_2$, weighed, and the apparent moisture content computed. The oven was closed immediately after the removal of each set of samples, and in computing the time of subsequent withdrawals, ten minutes was allowed for re-evacuation each time the oven was opened. The results obtained are also shown in Fig. 3 (dotted line).

The results of the first experiment show that the two samples of lowest initial moisture content had reached constant weight, over a two-hour interval, after 11 hours' heating, while the samples of high moisture content lost weight throughout the entire 20-hr. drying period. In the second experiment, in which selected samples only were removed at each time interval, and the oven immediately closed and evacuated, constant weight was reached in an 11-hr. heating period. The procedure used in the second experiment was doubtless more accurate and representative of the conditions employed in making moisture determinations. As a result of this study a 16-hr. drying period was employed throughout the comparative studies.

*Grinding of samples for 130° C. air oven*

The rapid air oven method for grain, as described in the official methods of the Association of Official Agricultural Chemists (9) requires the heating of the material for two hours in an oven at 135° ± 2° C. It is not stated how the material is to be ground. This Association and the American Association of Cereal Chemists both recommend drying for one hour at 130° ± 3° C.

in an air oven as a rapid method for flour. The air oven method is not recognized as an official one for grain, but according to Coleman (6) it is commonly employed. Determination of its suitability for this purpose therefore was one of the objects of this investigation.

As this method is to be employed as a rapid determination it is obviously out of the question to use the two-stage drying procedure employed with the vacuum oven samples. This again brings up the problem of grinding the grain, especially when damp. Furthermore, since the method was originally developed for flour it might be expected to be applicable only to finely ground material.

This method was therefore subjected to a preliminary experiment using further portions of the samples employed in the preliminary tests with the vacuum oven. Both the Hobart and Wiley grinders were used in combination with single- and two-stage drying methods. The results obtained are shown in Table II.

In these few preliminary experiments the air oven and the vacuum oven methods gave similar results on material treated in the same way. As the two-stage procedure is not permissible for a rapid method, the higher moisture contents indicated by it are of little consequence, except that one can predict that the rapid air oven method (single-stage) will give lower results than a two-stage vacuum oven method. The findings are indefinite as to whether the Wiley mill or Hobart grinder is the better for the 130° C. air oven method when the samples are ground directly. It was decided therefore to grind one half of the main series of 300 hard red spring wheat samples, referred to earlier, in the Wiley mill and the other half in the Hobart grinder, thus enabling the errors inherent in both methods to be determined as well as permitting comparisons with the vacuum oven results.

## Technique Finally Adopted

### *Vacuum oven*

As a result of the preliminary experiments already described, a two-stage method of drying was adopted. The vacuum oven technique employed was that described in the official methods (1, 9), using a 16-hr. drying period at a temperature of 99 to 100° C. and a pressure of 25 mm., or less, of mercury. Two De Khotinsky vacuum ovens, the vacuum chambers of which are immersed in oil, were employed, each oven being provided with a Hyvac pump. Metal dishes with inside fitting covers as required in the official methods (1) were used, the covers being loosened but not removed while drying proceeded in the oven. Calcium carbide was used in the desiccators in which the dishes were cooled.

### *Air oven*

No preliminary drying was employed in this determination. The ground sample was dried at 130° C. ± 3° C. for one hour, the official method for flour (1, 9). A size *C* De Khotinsky drying oven was used. Twenty dishes were placed in the oven at a time, these being all on one shelf. The thermometer bulb was at the same level as the dishes. Dishes with inside fitting

covers were employed, the covers being entirely removed during drying. Approximately 2-gm. samples were employed. The desiccators used were charged with calcium carbide.

*Brown-Duvel*

The Brown-Duvel tester has been fully described by Coleman and Boerner (7), but as special refinements are practised in the Dominion Grain Research Laboratory, where these tests were made, and in the offices of the Western Grain Inspection Division generally, the conditions found necessary to secure uniformity of operation will be outlined in some detail.

In accordance with the customary procedure, all thermometers, distillation flasks, measuring cylinders and condensing tubes were checked for accuracy of dimensions as specified by Coleman and Boerner (7), and in addition the thermometers were standardized between 160° C. and 195° C. by the Division of Physics, National Research Council of Canada. The calibrations of the measuring cylinders were checked within the range 10.5 to 17.0 cc. and those with an error greater than $\pm$ 0.1 cc. were discarded.

It has been found in the Dominion Grain Research Laboratory that the time required for heating and cooling has a decided effect on the results. The tester used was a six compartment machine manufactured by the Seed Trade Reporting Bureau, Chicago, but the heating elements were made in Winnipeg, to specifications prepared by the Laboratory. These required the individual heaters to give 675 $\pm$ 7 watts at normal line voltage. A separate transformer and heavy leads were used to prevent a voltage drop of over 2% when the testers were all in operation. Voltage controllers are employed at many of the Western Grain Inspection offices, but these were not installed in connection with the equipment used in this investigation. The line voltage, however, was checked frequently during the tests by means of a voltmeter mounted permanently above the tester. The heating rate in all compartments, as determined by the oil test, was finally rendered uniform by adjusting the distance of the heating element from the flask. The heating time employed for the oil test was 25 min. rather than 20 min. as specified by Coleman and Boerner.

In order to obtain a uniform cooling time the tester was located in a position protected from draughts, in a room held at approximately 21° C. by means of a thermostat. As the cooling time is also affected by the distance the heating plate falls away from the heating chamber, a set screw was provided on the swivel arm to regulate this distance. All compartments were thus adjusted so as to cool the flasks to 160° C. in approximately 20 min. when testing wheat of 14.5% moisture. Determinations on actual samples under examination were only made after the machines had been put into condition by a preliminary test. The oil used was at room temperature and sufficient was maintained to accommodate a full day's test, in order to avoid the use of warm oil returned from the tests to storage. The "cut-off" temperatures employed were as follows: hard red spring wheat 180° C.; amber durum wheat 180° C.; barley 190° C.; and oats 195° C.

## 4. Vacuum Oven Results

HARD RED SPRING WHEAT

Two of the subsamples, obtained from the subdivision of each sample, were dried to equilibrium at 72° F. and 40% relative humidity, and ground. Duplicate 2-gm. portions were taken from each for the vacuum oven determinations. Comparison of the results obtained from the two subsamples made possible an estimate of the sampling error.

As two ovens were required to accommodate the number of samples tested daily, a study of the difference between ovens was made possible, by placing one of the duplicates of each subsample in each of the two ovens. The two De Khotinsky vacuum ovens already described were operated under exactly the same conditions of temperature and pressure.

The 1200 individual results obtained from the three hundred wheat samples were subjected to an analysis of variance, the total variance being divided into portions attributable to average difference between ovens, differences between subsamples and the difference between duplicates after eliminating any average difference between ovens. The need for determining the sampling error in an investigation of this type was demonstrated by the fact that the variance between subsamples was significantly greater than that between duplicates of the same subsample, even in this relatively simple sampling process, which merely consisted of subdividing a small sample. There was also a significant difference between ovens, which demonstrates that ovens of identical construction, operated under the same conditions, may give slightly different results. The results of the foregoing analysis, expressed in terms of percentage of moisture, are summarized in Table III.

TABLE III

RESULTS OF ANALYSIS OF VARIANCE OF VACUUM OVEN DETERMINATIONS

| Grain | Statistic | Moisture, % |
|---|---|---|
| Hard red spring wheat | Average difference between ovens | 0.071 |
| Hard red spring wheat | Standard error of duplicates ("experimental error")* | 0.044 |
| Hard red spring wheat | Standard error of sampling | 0.110 |
| Hard red spring wheat | Standard error of duplicates plus sampling error* | 0.118 |
| Hard red spring wheat | Day-to-day variations (standard deviation) | 0.084 |
| Amber durum wheat | Standard error of duplicates plus sampling error* | 0.093 |
| Barley | Standard error of duplicates plus sampling error* | 0.089 |
| Oats | Standard error of duplicates plus sampling error* | 0.109 |

**Standard errors apply to mean of duplicates.*

Two subsamples of a large sample in each of the straight, tough, and damp classes, were analyzed daily by all methods, as previously mentioned, with a view to ascertaining whether the methods exhibited daily variations.

As the experiments with wheat were continued over a period of eleven days, the results should give a fairly reliable indication of the variations that might be encountered in practice. The daily fluctuation of the observed vacuum oven moisture content of each sample is shown graphically in Fig. 4.

The correlation between fluctuations in apparent moisture content of all three samples from day to day, is evident from the diagram, and is confirmed by an analysis of variance, which shows that the variation due to daily fluctuation, common to all three samples, is significantly greater than the residual variance attributable to sampling errors, experimental errors, etc. After allowing for these errors, the net variance attributable to daily variation corresponds to a standard error of 0.084% moisture, which is greater than the standard error of duplicates given in Table III.

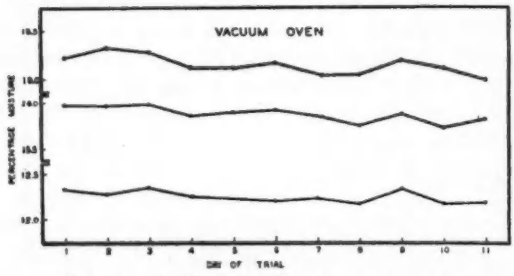


FIG. 4. *Daily fluctuation in moisture content by vacuum oven method (hard red spring wheat).*

### DURUM WHEAT, BARLEY AND OATS

The vacuum oven experiments with these grains were conducted in exactly the same manner as were those with hard red spring wheat, and the results were subjected to the same statistical analysis. Instead of 300 samples of each, however, only about 50 samples of amber durum wheat, 75 of barley, and 75 of oats were available. Towards the close of this phase of the investigation, it was found that the results secured from one of the ovens were affected by the position of the sample in the oven, portions of samples placed on the bottom shelf showing a moisture content as much as 0.25% lower than further portions of the same samples placed on the top shelf. The results obtained from this oven had, therefore, to be discarded. As two out of the four replicates of each sample were thus lost, it was impossible to separate the standard error of the duplicates from the sampling error, an estimate of the combined errors only being obtained. The standard error of the duplicates obtained from spring wheat may, however, with reasonable assurance be applied to these grains. The combined sampling and experimental errors for the different grains are given in Table III.

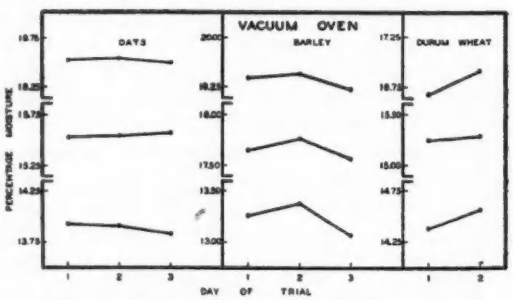


FIG. 5. *Daily fluctuation in moisture content by vacuum oven method (durum wheat, barley and oats).*

As the number of samples of durum wheat, barley and oats was much smaller than that of common wheat, the tests with any one grain were completed within two or three days. The moisture content of the three samples tested daily by the vacuum oven is shown in Fig. 5. There is some evidence of a systematic daily fluctuation in the results with barley and durum wheat, but the number of days available is too small to obtain a reasonably reliable estimate of the variance.

## 5. Air Oven Results

HARD RED SPRING WHEAT

It was felt at the outset that the 130° C. air oven would probably be the most accurate of the rapid methods, and in order to avoid a sampling error, possibly large enough to vitiate an estimate of its accuracy in comparison with the vacuum oven, the portions required for the former method were taken from one of the subsamples subsequently employed for the two-stage

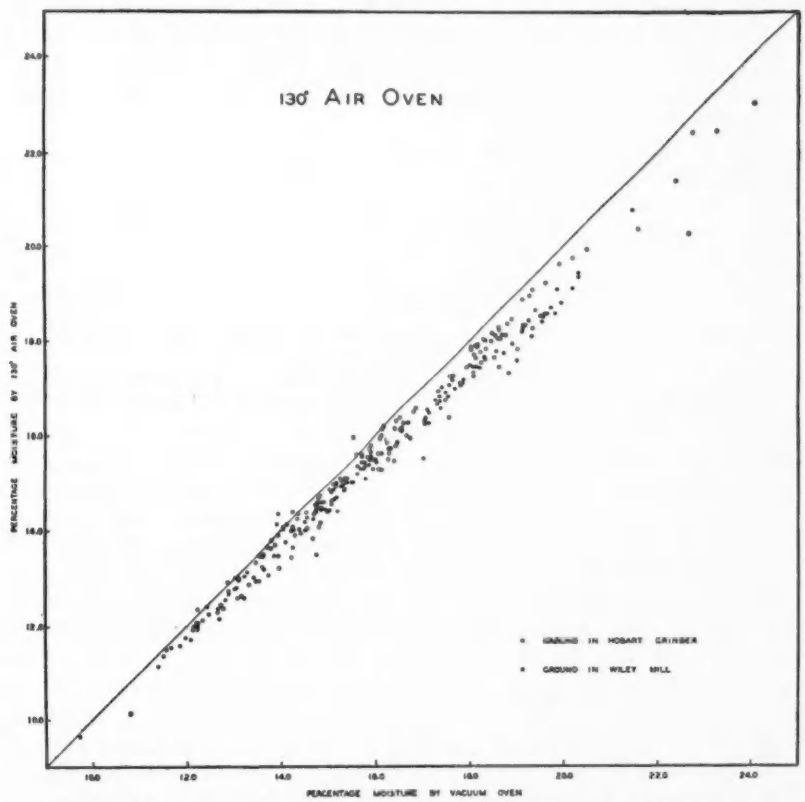


FIG. 6. *Moisture content of hard red spring wheat samples by 130° C. air oven and vacuum oven methods.*

vacuum oven determinations. Statistical comparisons were thus made between the air oven and the vacuum oven on the same subsample, so that the sampling error was virtually eliminated.

As previously mentioned, it was impossible to decide from the preliminary experiment whether the Hobart grinder or Wiley mill was best suited for the air oven method and in consequence 150 of the 300 wheat samples were ground in the Hobart and the other 150 in the Wiley mill.

The results obtained by both methods are shown in Fig. 6, from which it is evident that the Wiley mill has given more consistent results than the Hobart grinder. It is also evident that the air oven method, regardless of the

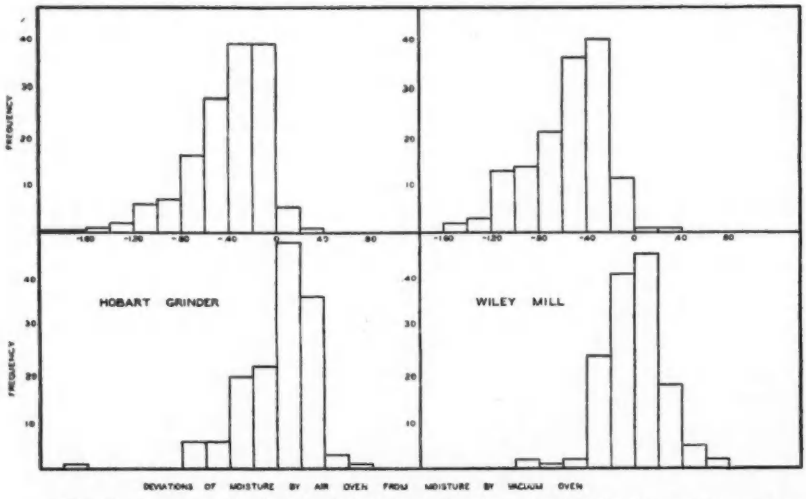


FIG. 7. *Frequency distribution of deviations of moisture by 130° C. air oven from moisture by vacuum oven (hard red spring wheat). Upper portion, observed deviations; lower portion, deviations after application of correction equation.*

method of grinding employed, consistently underestimates the amount of water present, and that the extent of this underestimation increases with the absolute moisture content. The results already presented in Table II suggest that the consistent underestimation of the moisture by the air oven can be attributed to the single-stage method of drying. This may also account in part for the increasing deficit with damp samples, although it is conceivable that the air oven is incapable of removing all of the moisture from such samples in the one-hour drying period.

The frequency distribution of the deviations of the observed moisture content by the air oven method, from the corresponding vacuum oven result, is shown for both methods of grinding in the top two histograms of Fig. 7. A distinct skewness is evident; most of the deviations are on the negative side, some being in defect by as much as 1.6% moisture. This systematic bias was eliminated by calculating by the method of Least Squares, a linear

regression equation expressing the expected vacuum oven moisture in terms of the observed air oven result, for both methods of grinding. These equations, together with the standard error of duplicates, the standard error of prediction of the vacuum oven values after applying the correction equations, and the net standard error of prediction due to differences in the results by the two methods, after allowing for the observational error in the vacuum oven determination, are shown in Table IV. In these equations the regression coefficients, by which the air oven results must be multiplied, give a measure of the tendency toward greater underestimation as the moisture content increases. The frequency distributions of the deviations of the air oven from the vacuum oven results, after applying this linear correction, are shown in the lower half of Fig. 7. It is evident that these distributions are now much more symmetrical, with the mode quite close to the zero value. The determinations made on material ground in the Hobart grinder, however, still exhibit a greater degree of asymmetry than those employing the Wiley mill.

TABLE IV

STANDARD ERROR OF DUPLICATES, LINEAR CORRECTION EQUATIONS AND OBSERVED AND NET STANDARD ERRORS OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE CONTENT OF HARD RED SPRING WHEAT BY THE 130° C. AIR OVEN USING TWO GRINDING METHODS

| Grinder employed | Standard error of duplicates | Correction equation* | Standard error of prediction | |
|---|---|---|---|---|
| | | | Observed | Net |
| Hobart | 0.069 | $M_V = -0.644 + 1.066\ M_A$ | 0.31 | 0.30 |
| Wiley | 0.050 | $M_V = -0.926 + 1.095\ M_A$ | 0.25 | 0.24 |

*$M_V$ = *moisture content as determined by vacuum oven.*
*$M_A$ = *moisture content as determined by air oven.*
*Standard errors apply to mean of duplicates.*

The standard error of the duplicates, commonly taken as a measure of the "experimental error" in the air oven method was higher than the value found for the vacuum oven (0.044%) by both methods of grinding, the Hobart grinder giving a larger error than the Wiley mill. It is noteworthy, however, that compared with this value the standard error of prediction of vacuum oven moisture by the air oven method is relatively large. This indicates that although duplicates may check fairly well within themselves, there may be a relatively large discrepancy in predicting the moisture content by the vacuum oven method from results obtained in the air oven. The net standard error of prediction, after allowing for the variance of duplicates in the vacuum oven, is not significantly smaller than the observed standard error. This is to be expected since the air oven and vacuum oven determinations were made on the same subsamples, as explained earlier, and the results are not affected by differences between subsamples. The results in Table IV show that the standard errors of prediction, and of the duplicates, are lower with the Wiley than with the Hobart grinder, showing the former mill to be superior for this determination.

The results obtained from the three samples tested daily by the air oven method to detect the existence of daily variation are shown in Fig. 8 for both methods of grinding. Upon analysis these do not reveal consistent daily fluctuations in the results obtained from all three samples. Nevertheless, in view of the known existence of daily variation in the vacuum oven results, it seemed desirable to investigate the effect of eliminating daily differences. These differences in both the vacuum and air oven results may be eliminated from comparisons of the two methods by taking the variances and covariance about the daily means, instead of about the general mean of the entire series. When this is done the standard error of prediction, using the Wiley mill, is reduced from 0.24% to 0.23% moisture, but the standard error of prediction, using the Hobart grinder, previously most variable, is reduced from 0.30% to 0.22% moisture. The reason for this reduction is apparent in Table V, which shows the average difference between the moisture determinations by the air oven and vacuum oven on different days. The Hobart grinder exhibits a variation in this respect of no less than 0.52% moisture as compared with 0.29% in the case of the Wiley mill, and a maximum difference of 0.24% in the daily series of vacuum oven results (Fig. 4). The observed irregularity of the Hobart results (Fig. 6) would thus appear to be due to the difficulty of maintaining an identical setting of the machine from day to day. The elimination of daily variations by the above method reduces the errors of prediction, but this lower value is only applicable to comparisons of the two methods on a particular day. The errors of prediction, given in Table IV, are those applicable to the air oven method with hard red spring wheat, as used in ordinary practice.

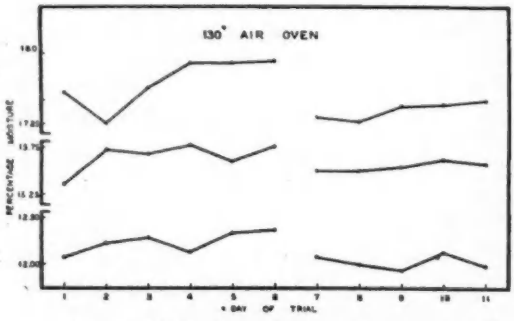


FIG. 8. *Daily fluctuation in moisture content by 130° C. air oven method (hard red spring wheat). Days 1–6, Hobart grinder; days 7–11, Wiley mill.*

TABLE V

AVERAGE DIFFERENCE BETWEEN PERCENTAGE MOISTURE BY 130° C. AIR OVEN AND MOISTURE BY VACUUM OVEN BY DAYS

| Day | Hobart grinder | Day | Wiley mill |
|---|---|---|---|
| 1 | −0.72 | 7 | −0.59 |
| 3 | −0.55 | 8 | −0.63 |
| 4 | −0.46 | 9 | −0.61 |
| 5 | −0.22 | 10 | −0.34 |
| 6 | −0.20 | 11 | −0.56 |

*Effect of grade*

Only a small number of low-grade samples was encountered. Thirty-seven of these, grading No. 4 Northern, No. 5, No. 6 or Feed, were selected for comparison with an equal number of high-grade (No. 1 Hard and No's 1, 2 or 3 Northern) samples of approximately the same moisture content. Owing

to the use of two grinders, and the pronounced day-to-day variations affecting results obtained when the Hobart grinder was used, valid comparisons could be made only between tests performed on the same day. The variance and covariance of vacuum oven and air oven moisture about the daily means were computed for high- and low-grade samples separately and collectively. An analysis of variance indicates that neither the regression of vacuum oven moisture on air oven moisture nor the standard error of prediction differed significantly in the two cases.

*Results with artificially dampened samples*

In view of the length of time elapsing between the tempering of the artificially dampened samples and the performance of the tests it seemed unlikely that these would be differentiated in any way from naturally damp samples. Nevertheless, previous findings by Coleman (6) indicated the desirability of comparing the behavior of artificially dampened samples in the electrical moisture meters with that of naturally damp grain, and this study was extended to include all methods. Somewhat surprisingly there did appear to be a significant difference with the air oven, the underestimation of the moisture being less with artificially dampened samples by about 0.25% moisture on the average. The majority of the naturally damp samples available were, however, tested on one day, on which there were no artificially dampened ones; and when the straight and tough samples, tested on the same day, were compared with the remainder of the straight and tough, these also exhibited a significant differentiation in the same direction. It appears likely, therefore, that the observed difference between natural and artificially dampened samples may be due partly, if not wholly, to daily fluctuations, of the type previously noted in the results by the vacuum oven, air oven, or both.

### Durum Wheat, Barley and Oats

The investigation of the air oven method with these grains was conducted in exactly the same manner as that employed with hard red spring wheat; the air oven sample being obtained from one of the vacuum oven samples to avoid the sampling error. As the Wiley mill gave the lowest error of prediction with spring wheat this grinder was the only one employed in connection with these other grains.

The results obtained by the air oven as compared with the vacuum oven for all three grains are shown in Fig. 9. It is obvious that they exhibit the same characteristics as those obtained with spring wheat, namely, a consistent underestimation of the moisture content which increases as the moisture increases. This systematic bias was eliminated, as before, by calculating in the same manner a linear regression equation for each of the three grains. The standard error of prediction of the observed vacuum oven values was computed, and also the error of prediction after eliminating the "experimental error" of the vacuum oven determinations. As one pair of duplicates was lost owing to the failure of the vacuum oven, only the combined error of the vacuum oven and the sampling error was obtained, so that in making this

correction, where no sampling error is involved, it was necessary to use the "experimental error" of the vacuum oven obtained from the results with hard red spring wheat. The formulas for the linear correction equations, the standard errors of prediction and the standard errors of duplicates, are given in Table VI for the three grains. The regression coefficients are again

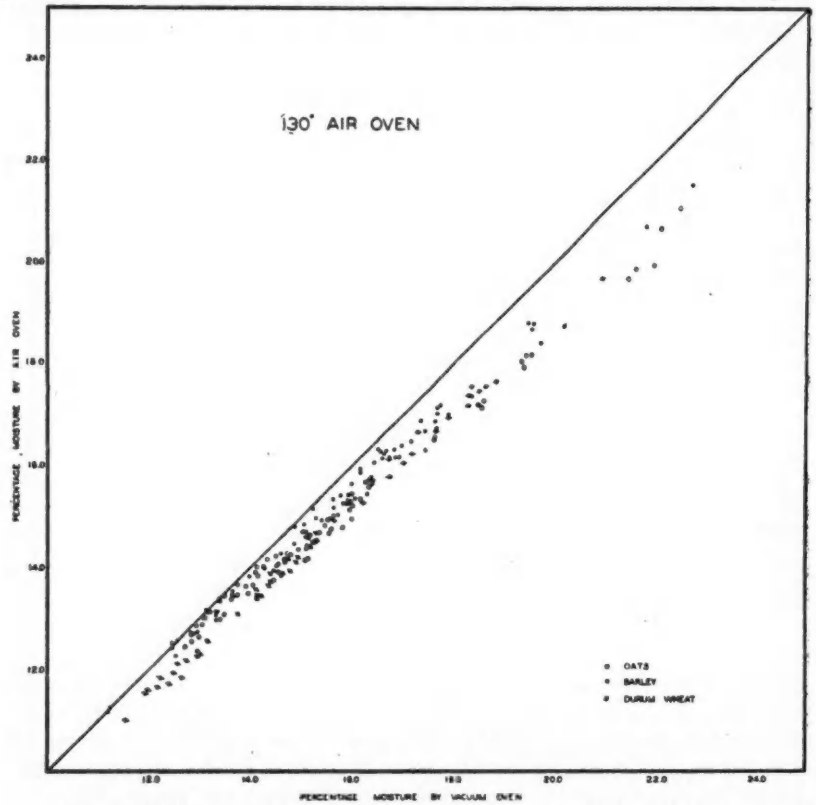


FIG. 9. *Moisture content of durum wheat, barley and oat samples by 130° C. air oven and vacuum oven methods.*

TABLE VI

STANDARD ERROR OF DUPLICATES, LINEAR CORRECTION EQUATIONS, AND OBSERVED AND NET STANDARD ERRORS OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE OF AMBER DURUM WHEAT, BARLEY AND OATS, BY THE 130° C. AIR OVEN

| Grain | Standard error of duplicates | Correction equation* | Standard error of prediction | |
|---|---|---|---|---|
| | | | Observed | Net |
| Amber durum wheat | 0.052 | $M_V = -0.452 + 1.084\ M_A$ | 0.13 | 0.12 |
| Barley | 0.056 | $M_V = -1.548 + 1.131\ M_A$ | 0.21 | 0.20 |
| Oats | 0.057 | $M_V = -2.225 + 1.195\ M_A$ | 0.21 | 0.20 |

*$M_V$ = *moisture content as determined by vacuum oven.*
*$M_A$ = *moisture content as determined by air oven.*
*Standard errors apply to mean of duplicates.*

all greater than unity, since this method shows an increasing tendency to underestimate the moisture content as the actual water content increases.

It is evident from Table VI that the standard error of the duplicates ("experimental error") is about the same for all three grains, being only slightly larger than that given in Table IV for common wheat ground in the Wiley mill. The standard error of prediction for all of these grains, however, is lower than the corresponding value for hard red spring wheat. There are two possible explanations for this behavior: either the air oven method is capable of more nearly reproducing the vacuum oven results with the other grains than with spring wheat, or else the analysts became more skilled during the course of the investigation, since these grains were studied last. It seems improbable, however, that the latter possibility accounts for the entire difference, since all of the men were skilled analysts, and since a similar result was observed with the Brown-Duvel method, the operator of which had been making the determination continuously for years.

The results obtained from the amber durum, barley and oat samples employed in the study of daily variations are shown in Fig. 10. As fewer samples of these grains were tested, than in the case of hard red spring wheat, the results do not extend over a period of more than three days at the most. Again the three individual samples yield no conclusive evidence of daily variation, but as before, the situation with respect to the results as a whole was investigated by computing the variances and covariance around the daily means instead of the general mean. When this was done the standard error of prediction for amber durum wheat was unchanged, that for barley was reduced from 0.20% to 0.15% and that for oats from 0.20% to 0.17%. These results demonstrate the extent to which daily variations affected the comparisons of the two methods. The larger error is the one applicable to ordinary routine determinations.

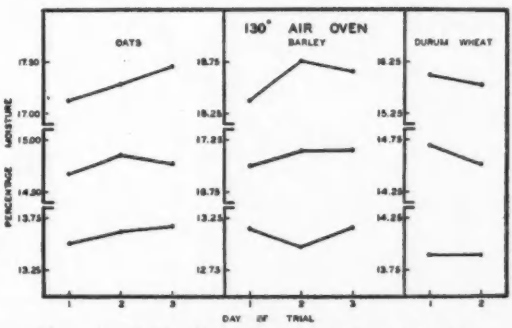


FIG. 10. *Daily fluctuation in moisture content by 130° C. air oven method (durum wheat, barley and oats).*

## 6. Brown-Duvel Results

HARD RED SPRING WHEAT

The Brown-Duvel determination was made according to the technique previously described, using a separate subsample of grain. In comparing this method with the vacuum oven, allowance must be made for both the experimental error of the vacuum oven determination and errors of sampling.

The results obtained by the Brown-Duvel method are plotted against the corresponding vacuum oven results in Fig. 11. They show essentially the same behavior as did the air oven results, namely, an underestimation of the moisture content which increases as the moisture increases. In this instance, however, the phenomenon is believed to be due to an entirely different set of causes, since the grain had not been ground. The consistent

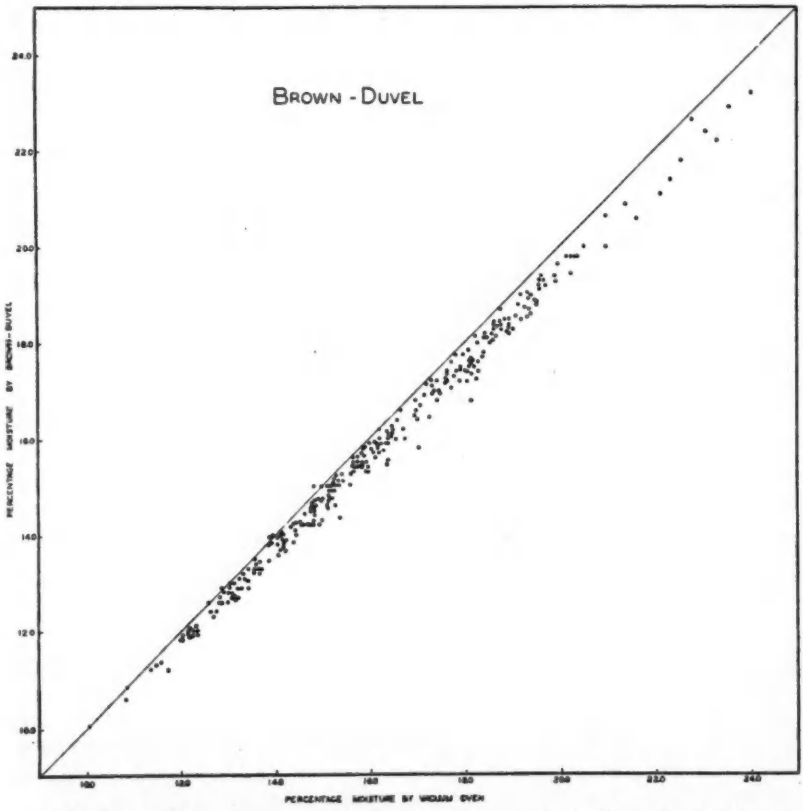


FIG. 11. *Moisture content of hard red spring wheat samples by Brown-Duvel and vacuum oven methods.*

underestimation of the moisture content is easily explained by the fact that the Brown-Duvel method was originally developed to give results comparable with the old water-jacketed oven drying method, which in turn is known to give lower moisture values than the vacuum oven. It is also known (1) that a cut-off temperature of 190° C. gives moisture values more nearly equivalent to those of the vacuum oven than a cut-off temperature of 180° C. which is the one commonly used for wheat, and employed in this investigation.

Although the consistent underestimation by the Brown-Duvel method may thus be readily accounted for, these considerations can scarcely explain

why the extent of the underestimation should become progressively greater as the moisture content increases. This may be due to variation in the heating time with grain of different moisture content. It has been shown by Coleman and Boerner (7) that slow heating gives low results while rapid heating gives high results. As the time required to attain the desired temperature increases with the moisture content of the grain, even when the heaters are carefully standardized by the oil test, it is obvious that a differential deficit is to be expected.

The frequency distribution of the deviations of the observed moisture content by the Brown-Duvel from that by the vacuum oven method is shown in the upper part of Fig. 12. A distinct negative bias is observable, but on the whole the deviations show a more symmetrical distribution than those of the air oven results. This systematic bias was eliminated by computing a linear regression equation as before. This equation, together with the standard error of prediction of the observed vacuum oven values and the standard error of prediction after allowing for the sampling error and experimental error of the vacuum oven method are given in Table VII. The frequency distribution of the deviations after applying the correction equation is shown in the lower part of Fig. 12. The increase in accuracy is evident.

Table VII shows that the standard error of the duplicates in the Brown-Duvel method is practically the same as that obtained using the Wiley mill in conjunction with the air oven, these being respectively 0.053% and 0.050%. The standard error of prediction of the vacuum oven results, however, is much lower for the Brown-Duvel than for the Wiley mill and the air oven. The net standard error of prediction attributable to discrepancies between the methods is much less than the observed error, owing to the fact that the latter includes errors of sampling.

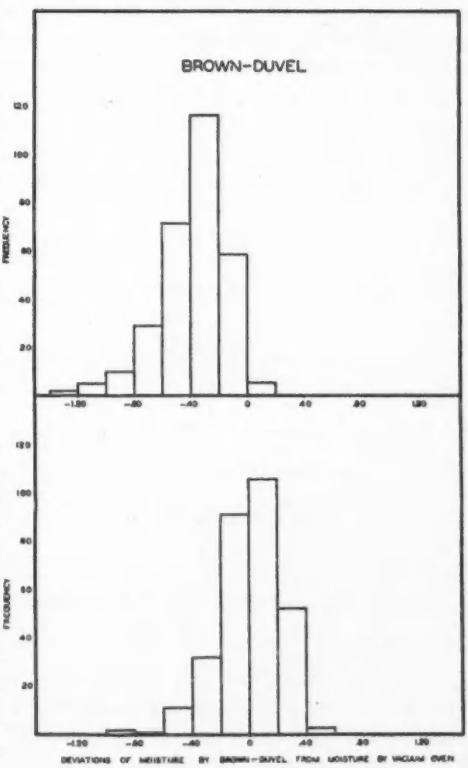


FIG. 12. *Frequency distribution of deviations of moisture by Brown-Duvel from moisture by vacuum oven (hard red spring wheat). Upper portion, observed deviations; lower portion, deviations after application of correction equation.*

TABLE VII

STANDARD ERROR OF DUPLICATES, LINEAR CORRECTION EQUATIONS, AND OBSERVED AND NET STANDARD ERRORS OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE BY THE BROWN-DUVEL METHOD WITH HARD RED SPRING WHEAT, AMBER DURUM WHEAT, BARLEY AND OATS

| Grain | Standard error of duplicates | Correction equation* | Standard error of prediction | |
|---|---|---|---|---|
| | | | Observed | Net |
| Hard red spring wheat | 0.053 | $M_V = -0.280 + 1.042M_{BD}$ | 0.21 | 0.16 |
| Amber durum wheat | 0.040 | $M_V = 1.026M_{BD}$ | 0.15 | 0.09 |
| Barley | 0.042 | $M_V = 0.046 + 1.005M_{BD}$ | 0.17 | 0.12 |
| Oats | 0.034 | $M_V = 0.674 + 0.983M_{BD}$ | 0.16 | 0.13 |

*$M_V$ = *moisture content as determined by vacuum oven.*
*$M_{BD}$ = *moisture content as determined by Brown-Duvel.*
*Standard errors apply to mean of duplicates.*

These results show that the Brown-Duvel method, when used by a skilled operator and all precautions are taken to ensure accuracy, is superior to the air oven method, using the best grinder tested, for estimating the moisture content of hard red spring wheat as determined by the vacuum oven method. Both methods, however, underestimate the moisture content, but for different reasons, and the application of a linear correction equation is required to estimate the true moisture content as determined by the vacuum oven method.

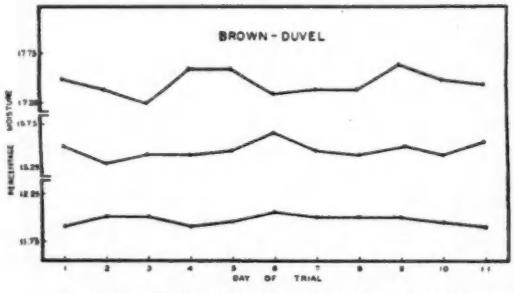


FIG. 13. *Daily fluctuation in moisture content by Brown-Duvel method (hard red spring wheat).*

The three samples of common wheat tested daily by the Brown-Duvel method (Fig. 13) show no evidence of systematic daily fluctuation. Nevertheless the standard error of prediction was reduced from 0.16% to 0.13% moisture by calculating the variances and covariance around the daily means instead of the general mean, indicating that day-to-day variations have been a definite source of error in this comparison of the vacuum oven and Brown-Duvel methods.

*Effect of grade*

As in the case of the 130° C. air oven, the results obtained with the small number of low-grade samples encountered did not differ significantly from those of the high-grade samples selected for comparison.

*Results with artificially dampened samples*

With artificially dampened samples a similar method of analysis to that applied to the air oven observations again gave similar results, using the vacuum oven as standard. There appeared to be a significant difference

between natural and artificially dampened samples, but differences in the same direction were also obtained by contrasting the results with straight and tough samples, on the three days on which naturally damp samples occurred, with those of the remaining straight and tough samples. This occurrence is in fact common to all methods and instruments tested, strongly suggesting daily variations in the vacuum oven as the underlying cause.

### Durum Wheat, Barley and Oats

The results for all three grains are presented in Fig. 14. It is evident that the discrepancies between the vacuum oven and Brown-Duvel results are considerably smaller than those obtained with hard red spring wheat; durum wheat showing the greatest departures. The linear equations, calculated as before, for estimating the vacuum oven moisture value from the Brown-Duvel determination for each of the grains, are given in Table VII. Application of these correction equations eliminates the systematic bias inherent in

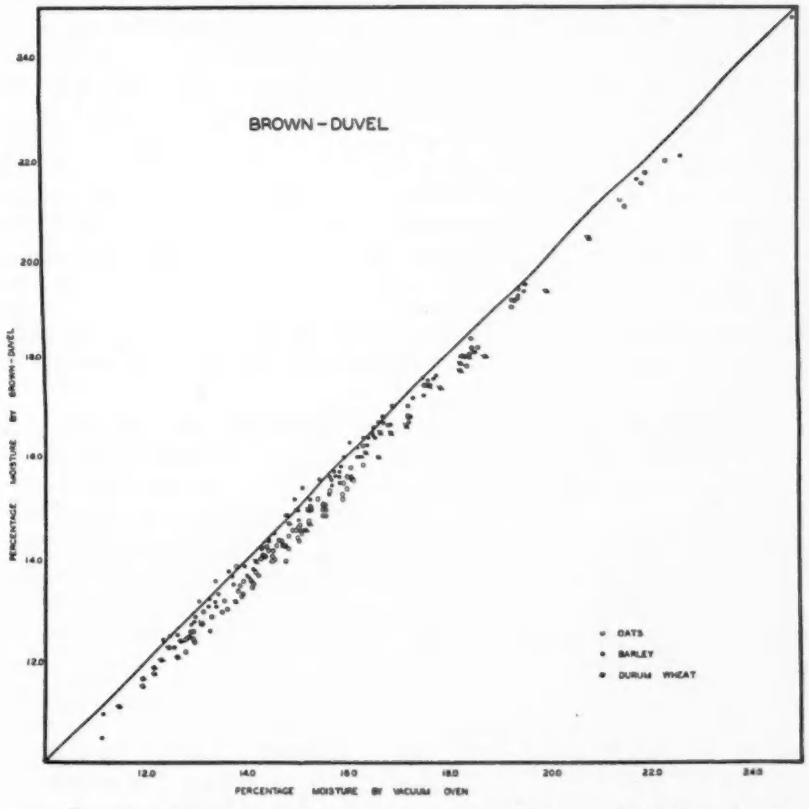


Fig. 14. *Moisture content of durum wheat, barley and oat samples by Brown-Duvel and vacuum oven methods.*

the method. The regression coefficient is in all cases close to unity, showing that this method maintains a practically uniform differential throughout the moisture range studied.

It would appear that the cut-off temperatures recommended for these grains yield results in closer agreement with the vacuum oven method than the cut-off temperature recommended for common wheat. The tendency of the method toward increased underestimation with increasing moisture content is evident only with durum wheat. As this behavior is not shown by oats and barley, it is possible that a variation in the heating time has less effect on the results with these grains. Presumably the presence of the hull on oats and barley removes part of the complicating effect of decomposition.

The observed standard errors of prediction of the vacuum oven results by the Brown-Duvel, and the net errors after allowing for the experimental error of the vacuum oven method and the sampling error, are given in Table VIII together with the standard errors of the duplicate determinations. Comparison of the errors of the duplicates with the corresponding values for common wheat shows that the Brown-Duvel determination is somewhat more accurate when dealing with the coarse grains. The standard error of prediction is likewise less than that obtained with hard red spring wheat, a result in harmony with the air oven findings. It appears therefore that both the air oven and Brown-Duvel are more accurate for coarse grains than for common wheat. Comparison of the air oven with the Brown-Duvel shows the latter to be superior for predicting the moisture content, as determined by the vacuum oven, in all grains tested. The "experimental error" of both methods is about the same in all cases, the Brown-Duvel being slightly better than the air oven with coarse grains. Both methods underestimate the moisture content and the results require correction by means of a linear regression formula to give results comparable with those obtained by the vacuum oven.

The results obtained from the three samples tested daily during the study of coarse grains are plotted in Fig. 15. These do not show any systematic daily variation, but when the variances and covariance of the experimental samples are calculated around the daily means instead of the general mean, the net standard error of prediction for durum wheat is reduced from 0.09% to within the sampling error, and for barley from 0.12% to 0.11%. The reduction in the case of oats affects only the third decimal place.

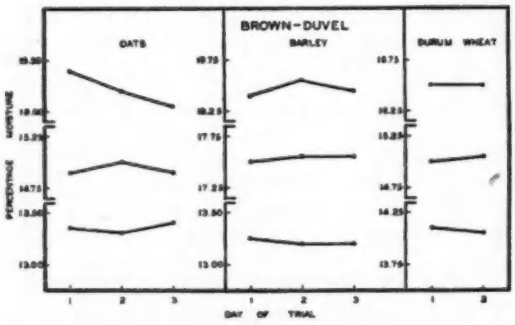


FIG. 15. *Daily fluctuation in moisture content by Brown-Duvel method (durum wheat, barley and oats).*

## 7. Summary and Conclusions

*Vacuum oven*

1. A double-stage drying procedure, in which wheat was dried to equilibrium with the room atmosphere before grinding, was found to give higher moisture values than those obtained when the grain was ground directly. The former procedure was therefore used in conjunction with the vacuum oven determinations.

2. When the vacuum oven used was filled with samples, at least 11 hours' heating was required for these to reach constant weight over a two-hour interval. A 16-hr. drying period was therefore employed for all experimental determinations.

3. Two vacuum ovens of identical construction, operated under the same conditions of temperature and pressure, gave an average difference of 0.071% moisture between 300 pairs of duplicate samples of hard red spring wheat, one oven yielding consistently slightly higher results than the other.

4. After eliminating the average difference between ovens the standard error of the mean of duplicates ("experimental error") was 0.044% moisture with hard red spring wheat and the daily fluctuations, computed from three pairs of samples tested daily, resulted in a standard deviation of 0.084% moisture. The experimental error thus forms only a part of the variance encountered in the vacuum oven method and is not an accurate measure of the precision of determinations made by it.

5. The relatively simple sampling operation of dividing a small amount of grain into eight equal subsections resulted in a sampling error of 0.110% moisture with hard red spring wheat. This was the limiting factor determining the precision of the vacuum oven, and the accuracy of the various rapid methods tested. It is evident that in investigations of this type, an estimate of the sampling error must be made if the correct conclusions are to be drawn from comparisons of different methods.

6. The combined experimental and sampling errors encountered with other grains were: amber durum wheat 0.093%, barley 0.089%, and oats 0.109%.

*130° C. air oven*

7. Both Hobart and Wiley grinders were employed in conjunction with the 130° C. air oven method; the latter was found to be more accurate as shown from the standard error of prediction given in Table VIII. The greater error with the Hobart grinder was traced to greater daily fluctuations.

8. The air oven method was found to underestimate the moisture content, as determined by the vacuum oven, of all grains studied, the deficit increasing with the actual moisture content. The consistent underestimation is believed to be due to evaporation during grinding, as in the former method the grain was ground without preliminary drying. This procedure may also account in part for the increase of the deficit with moisture content, but it is also conceivable that the air oven is incapable of removing all of the moisture from damp samples in a one-hour drying period.

*Brown-Duvel*

9. The Brown-Duvel method was also found to underestimate the moisture content of most of the grains studied; and with hard red spring and amber durum wheat, the deficit increased with the actual moisture content.

TABLE VIII

"EXPERIMENTAL ERROR" AND STANDARD ERROR OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE BY AIR OVEN AND BROWN-DUVEL METHODS

| Determination | Standard error of duplicates ("Experimental error") | Net standard error of prediction |
|---|---|---|
| Air oven | | |
| Hard red spring wheat | | |
| Hobart grinder | 0.07 | 0.30 |
| Wiley mill | 0.05 | 0.24 |
| Amber durum wheat (Wiley) | 0.05 | 0.12 |
| Barley (Wiley) | 0.06 | 0.20 |
| Oats (Wiley) | 0.06 | 0.20 |
| Brown-Duvel | | |
| Hard red spring wheat | 0.05 | 0.16 |
| Amber durum wheat | 0.04 | 0.09 |
| Barley | 0.04 | 0.12 |
| Oats | 0.03 | 0.13 |

*Standard errors apply to mean of duplicates.*

10. It is evident from Table VIII that the Brown-Duvel method is capable of giving a more consistent estimate of the moisture content as determined by the vacuum oven, than is the 130° C. air oven. As the Brown-Duvel determination is more dependent on the skill of the operator and on a close adherence to the specified conditions, than the air oven determination, this result might not apply in general practice, particularly if Brown-Duvel equipment operated with gaseous or liquid fuel had to be used. The above tests, however, can be taken as typical of the Brown-Duvel determination as used in the offices of the Western Grain Inspection Division.

11. The standard errors of prediction given in Table VIII indicate that the air oven and Brown-Duvel methods are capable of greater accuracy with coarse grains than with wheat.

*Daily fluctuations*

12. The wheat samples analyzed daily to study the extent of the daily fluctuations in methods, show systematic variations in the vacuum oven, and in the air oven when the material was ground in a Hobart grinder. The results obtained by the remaining methods with wheat and with the coarse grains by all methods, did not in general give clear-cut results, but the standard errors of prediction were in most cases reduced by calculating the variances and covariance around the daily means. This method of computation removes any bias due to daily fluctuations in either the vacuum oven or the method under test. It seems probable, therefore, that small daily variations

occurred in several of the series of tests, but owing to the greater residual variation they could not be demonstrated as clearly as in the case of the vacuum oven.

## Acknowledgments

The authors wish to acknowledge the ready assistance of the staffs of the Western Grain Inspection Division, who collected and cleaned the samples, and in particular that of Messrs. W. Chivers and R. Forrester of the Winnipeg Inspection Office, who subdivided the samples. Acknowledgment is also due to Mr. B. Frisell, Mr. F. H. Lehberg and Mr. A. T. Capewell of the Dominion Grain Research Laboratory, who were responsible for the vacuum oven, air oven and Brown-Duvel determinations respectively. Mr. S. Stein, Research Assistant, Associate Committee on Grain Research, ably assisted with the various oven determinations.

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# THE CANADIAN SPECIES OF THE TORTRICID GENUS *PERONEA*[1]

By J. McDunnough[2]

## Abstract

Forty-three species of the Tortricid genus *Peronea* are dealt with, comprising all the species known to occur in Canada at the present time and including practically all of the North American ones. Characters found in both the male and female genital organs have been used as a means of specific differentiation and there are eight sets of figures illustrating these organs. The range of variation in color and pattern of the individual species is briefly discussed and records of distribution and larval food-plants are given as far as present knowledge permits. Seven species new to science are described.

## Introduction

The numerous North American species of the Tortricid genus *Peronea* have long been in need of a thorough revision. Owing to the extraordinary variability of color and pattern in the individual species and the superficial similarity of distinct species, misidentifications have been the rule rather than the exception; to increase the difficulties a number of species are common to both the Nearctic and Palaearctic regions and the attempts to apply the older and valid European names to our North American forms have often resulted disastrously, and increased, rather than improved, the synonymic tangles.

It is only of recent years, thanks to the studies of Pierce, Filipjev, and Sheldon, that the Palaearctic species have become thoroughly understood, and these studies have definitely proved the enormous value of characters found in both the male and female genital organs as a means of delimiting the individual species and correctly associating the numerous forms, both of color and pattern, that have heretofore proved so perplexing. The present paper is an attempt to place our Nearctic, and more particularly our Canadian, species on a similar sound nomenclatorial basis, by the use of characters found in the structure of the genitalia. The material studied has been largely that contained in the Canadian National Collection, but the opportunity has also presented itself, through the courtesy of the collectors, of working over the specimens in the collections of Mr. K. Bowman of Edmonton, Alta., and Mr. W. R. Buckell of Salmon Arm, B.C., thereby increasing notably our knowledge of the range of distribution of a number of species. Considerable valuable material from the Kearfott collection was also made available for study through the co-operation of the authorities of the American Museum of Natural History in New York City, to whom sincere thanks are due. While it has been impossible in most instances actually to examine existing type material, every care has been used in making the specific identifications, based on a detailed study of the original descriptions and such figures as were available. In doubtful cases photographs of the types were secured or speci-

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*Contribution from the Division of Systematic Entomology, Entomological Branch, Department of Agriculture, Ottawa, Canada.*
[2] *Chief, Division of Systematic Entomology.*

mens sent for comparison to museum specialists, and in this connection the writer wishes to acknowledge gratefully the assistance of Mr. W. H. T. Tams of the British Museum of Natural History, Mr. A. Busck of the United States National Museum and Dr. E. T. Cresson of the Academy of Natural Sciences, Philadelphia. With one or two exceptions, which will be noted in the text, it is felt that the specific determinations are reasonably sure of being correct.

As the genus *Peronea* is comprised essentially of species indigenous to temperate zones, it has not been surprising to find that of all the described North American species, there are only two which as yet have not been found to occur in our Canadian fauna; these are *foliana* Wlshm., a species from the southwestern States and *comariana* Fern., a small species from the Atlantic States which is entirely unknown to the writer. Even with our present limited records the range of distribution of the individual species is very extensive and it is fairly safe to predict that most of the species will be found to extend in range across the entire Dominion.

No attempt has been made at this time to create names for any of the individual color forms and pattern variations, as has been done in Europe. The aim of this paper is merely to present constant structural characters whereby the species may be differentiated, and it is felt that the study of the variation within any one given species may be safely left for some future time when more material, especially in long bred series, is available for study. Further it has been found inadvisable to apply subgeneric names in the group as has been attempted by various European workers; the characters used to define these subgenera appear to the writer to be largely specific rather than subgeneric and several instances occur, as will be noted in the text, where a species links up with two so-called subgenera.

### ***Peronea emargana*** Fabr.

Page 325, fig. 1; Page 329, fig. 1.

*Pyralis emargana* Fabricius, Syst. Ent. 651, No. 37. 1775.
*Pyralis caudana* Fabricius, Syst. Ent. 651, No. 38. 1775.
*Acalla emargana* Kennel, Palaearct. Tort. 65, Pl. III, figs. 1-4. 1909.
*Rhacodia emargana* Pierce and Metcalfe, Genit. Brit. Tort. 23, Pl. IX. 1922; Sheldon, Entomol. LXIII, 121. 1930; Filipjev, Ann. Mus. Zool. Acad. Sci. U. R. S. S. 524, Pl. XXV, fig. 2, Pl. XXX, fig. 5. 1930.

The species is known in our North American lists under the name *caudana* Fabr. and is readily recognized by the strong excavation of the costal margin of the forewing. The writer has seen no Nearctic specimens of the typical form with yellow basal area on primaries, all our specimens being rather uniformly dark gray-brown or ruddy-brown with (in eastern Canadian specimens at least) a red streak along the inner margin; this, according to Sheldon, is typical *caudana*. While the genitalia of Canadian specimens do not match exactly with the figures given by Pierce, they do coincide much more closely with those of Filipjev and until, therefore, the matter can be straightened out by European students, it seems best to list the species under the above name.

European writers separate the species from *Peronea* under the generic term *Rhacodia* Hbn. on the strength of the slightly subfalcate forewings with excavated costal margin and in the genitalia, according to Filipjev, (*op. cit.* p. 501), the strong, apical, downward projection of the male valva and the narrow band-like nature of the signum in the female bursa. As regards these characters, the writer is of the opinion that they are of individual, rather than of generic, value; *maccana* has essentially the same type of male genitalia with the exception of a very different aedeagus, but has the normal wing-shape and has always been included in *Peronea*. Filipjev, it is true, suggests (p. 502) that a new subgenus may be needed for this species along with *schalleriana (logiana)* and *sponsana*, but both of the latter species show much more divergence in male genitalia from *maccana* than does *maccana* from *emargana*. Several other species following *maccana* in our present arrangement show more distant relationships with the *emargana* group, as can readily be seen by a reference to the figures. The safest plan, unless one is willing to create a multiplicity of doubtful subgenera, seems to be to include all species under the one generic heading, as noted in the introduction.

*Genitalia, Male* (Two slides examined). Tegumen without cristae; apical portion of anal sheath rather hood-like, with slight transverse ventro-apical projection; socii long, very narrow, upright; claspers strongly narrowed apically, sacculus bent upward toward costa beyond half, then recurved, ending bluntly in a strong hair-tuft, preceded by a deep excavation, apex of valvula long, pointed, extending above costal margin; aedeagus short, scarcely bent, broad at base, narrowed somewhat apically with a small ventro-apical spine, no cornuti.

*Genitalia, Female.* Ostium plate narrow, lateral lobes wide apart, moderately long, narrowed rather suddenly apically with small inner projection; ostium slightly asymmetrical, situated on cephalic margin of plate; ductus bursae entirely membranous, long, narrow; signum a small, narrow, scobinate strip of chitin.

*Distribution.* Ontario, Ottawa, Biscotasing; Alberta, Banff, Calgary, Nordegg; British Columbia, Victoria.

*Food Plant. Salix, Populus, Betula* (in Europe).

### *Peronea maccana* Tr.

Page 325, fig. 2; Page 329, fig. 2.

*Teras maccana* Treitschke, Schmett. Eur. X (3), 133. 1835.
*Acalla maccana* Kennel, Palaearct. Tort. 74, Pl. IV, figs. 19, 20. 1909.
*Peronea maccana* Pierce and Metcalfe, Genit. Brit. Tort. 21, Pl. VIII (*partim*, ♀ *nec* ♂). 1922; Sheldon, Entomol. LXIII, 222. 1930; Filipjev, Ann. Mus. Zool. Acad. Sci. U. R. S. S. 502. 1930.
*Teras fishiana* Fernald, Trans. Am. Ent. Soc. X, 66. 1882; Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 483. 1924.

A larger species than *hastiana* and readily separated by the very striking form of the male genitalia. Characteristic also is the rigidly oblique line from costa of forewings before half to inner margin at two-thirds, with its edging

of blackish, or reddish-black, slightly raised scales, separating the pale antemedian band from the outer area which is generally largely suffused with red-brown, purple-brown or black-brown, especially in costal section.

The type of clasper in the male genitalia is essentially that of *emargana* Fabr. and the socii also show a relationship; the aedeagus is however of a quite dissimilar type. There are a few very slight, minor differences between the genitalia of European and North American specimens, but scarcely sufficient to warrant the retention of the name *fishiana* Fern. even in a varietal sense.

*Genitalia, Male* (Two slides examined). Very large, with the claspers narrowed and bent inwards apically. Tegumen broad, without cristae; anal sheath narrowed apically with a strong, pointed, ventro-apical spine; socii long, narrow, erect; claspers narrowed apically, sacculus bent strongly upward toward costa at half, then just as strongly recurved forming a deep excavation and terminating in a curved, blade-like process, beyond which is a strong apical hair-tuft; apex of valvula long, conical, extending above costal margin; aedeagus very long and thin, vesica with a terminal bundle of about six minute cornuti.

*Genitalia, Female* (Two slides examined). Ostium plate with very broad, long, lateral lobes with a minute spine on each inner edge apically; ostium situated near centre of plate, narrow, semicircular; ductus bursae with the narrow, proximal, chitinized portion extending to just beyond the cephalic margin of ostium plate; membranous portion of ductus expanded proximally and with traces of irregular chitinization, then narrowing to a long, thin tube; bursa small, flask-shaped; signum minute.

*Distribution.* Quebec, Meach Lake, Aylmer; Ontario, Ottawa, Biscotasing; Alberta, Edmonton; British Columbia, Vancouver.

*Food Plant.* *Vaccinium* (in Europe).

### *Peronea ptychogrammos* Zell.

Page 325, fig. 6; Page 329, fig. 3.

*Teras hastiana* var. *ptychogrammos* Zeller, Verh. zool. bot. Gesell. Wien, 213. 1875.

This small species, confused by Zeller and all subsequent workers with *hastiana*, has in reality nothing to do with this species; on genitalic characters it is quite distinct and occupies a unique position in the genus with no very close allies; the claspers show certain similarities with those of *emargana* and *maccana* and for this reason it is given its present position.

The color of the forewings is light gray with a moderately distinct red-brown costal triangle; the most distinctive features of the maculation are a *small black basal dash* just below costa and fine black lines along the cubital and anal veins and along the inner edge of the costal triangle.

*Genitalia, Male* (Two slides examined). Tegumen broad apically and rather chunky; anal sheath unarmed, short; socii weak, small, roughly circular; sacculus lightly sinuate, terminating in a large raised circular tuft of coarse hairs, the inner edge of which is produced downward to form *a long sharp spine;* aedeagus short, thin, vesica armed with a stout apical spine and two pairs of very small cornuti.

*Genitalia, Female* (One slide examined). Ostium plate small, but with well-developed lateral lobes, directed slightly outward; ostium on cephalic margin of plate, straight; chitinized portion of ductus bursae short, rectangular; membranous portion enlarged somewhat proximally to a small sack which contains a lightly chitinized plate; signum hemispherical, scobinate.

*Distribution.* Manitoba, Aweme; British Columbia, Salmon Arm.

*Food Plant.* Unknown.

## *Peronea nigrolinea* Rob.

Page 325, fig. 3; Page 329, fig. 5.

*Teras nigrolinea* Robinson, Trans. Am. Ent. Soc. II, 281, Pl. VII, fig. 67. 1869.
*Teras ferruginiguttana* Fernald, Trans. Am. Ent. Soc. X, 65. 1882.

The name was based on several specimens from Grimsby, Ont. (Pettit); two of these, from the collection of the American Museum of Natural History, have been available for study through the courtesy of the authorities and one of them has been chosen as "lectotype" by the writer; four specimens from Ottawa in the Canadian National Collection match this type excellently. The species is a large one with light gray forewings strongly roughened with numerous scale-tufts; as stated by Robinson, the black line from base to apex of wing is not always sharply indicated, in fact, its presence is probably of rather rare occurrence.

In the western provinces a form with similar genitalia occurs in which the primaries are of a much deeper blue-gray color, variably mottled and suffused with smoky shades and frequently with small sub-basal and median dark patches in the fold; rarely slight ochreous or ruddy tinges occur near base and apex of wing. Following a recommendation by Mr. A. Busck of the United States National Museum, the name *ferruginiguttana* Fern. may be applied to this in a varietal or racial sense; Mr. Busck informs me that all that remained of the unique female type of *ferruginiguttana* from Colorado, when the Fernald collection was taken over, was a single hindwing. His conception of the species is based on other Colorado specimens which agree in genitalia with the form occurring in the western Canadian provinces. The male genitalia are extraordinarily dissimilar to those of any *Peronea* species except the following one; there is probably a certain affinity, in the shape of the claspers, to *maccana* Tr. and through this species to the *emargana* group, but this association is not confirmed by other details in these organs, notably the shape of the socii and aedeagus.

*Genitalia, Male* (Two slides examined). Tegumen broad, without cristae; anal sheath rather pointed at apex, without any definite armature; socii somewhat decumbent, roughly oval in shape; sacculus drawn out into a long point before half and then curving costad sharply and strongly, the apical section again bent downward and *bluntly truncate*, the large ventral excavation thus formed being roughly circular in outline; apical tuft strong, raised; apex of valvula projecting upward beyond margin of costa; aedeagus long, stout at base, then tapering, strongly bent; vesica armed with a large, stout apical spine and a cluster of four or five small cornuti.

*Genitalia, Female.* Ostium plate rather narrow, lateral lobes moderately long, wide apart and directed outward with a slight incurve at apex; ostium wide, sinuate; ductus bursae with the chitinous portion moderately long and *obliquely cut off* distally, with the inception of the ductus seminalis immediately beyond this portion; membranous section of ductus long, broad, of slightly varying width; bursa contracted somewhat before fundus with strong stellate signum.

*Distribution.* Typical form. Ontario, Ottawa, Grimsby. Var. *ferruginiguttana.* Manitoba, Aweme; Alberta, Whitla, High River, Edmonton; British Columbia, Salmon Arm.

*Food Plant.* Unknown; possibly *Populus.*

### *Peronea maximana* B. & B.

Page 325, fig. 4; Page 329, fig. 6.

*Peronea maximana* Barnes and Busck, Cont. Nat. Hist. Lep. N. Am. IV (3), 216, Pl. XXXII, fig. 1. 1920.

Very similar to the variety *ferruginiguttana* of the preceding species in size, shape and coloration of forewings. In general the forewings appear much smoother and are more evenly dark blue-gray with less smoky shading; they present further a slightly strigate appearance, due to the presence of very fine, broken dark lines on the veins; in one specimen there is a distinct dark streak from base to apex of wing.

*Genitalia, Male* (Two slides examined). Very similar in general characters to *nigrolinea.* Socii somewhat smaller; sacculus *truncate* at half, not drawn out to a long point; apex, however, produced to a *long curved spine,* with smaller hair-tuft; apex of valvula shorter; vesica with an increase in the number of small cornuti.

*Genitalia, Female* (One slide examined). Lateral lobes of ostium plate broader and closer together than in *nigrolinea;* ostium closer to caudal margin of plate and openly V-shaped; chitinized portion of ductus bursae very long, narrowed distally and with a V-shaped terminal incision.

*Distribution.* Ontario, Biscotasing, Bobcaygeon; Alberta, Edmonton; British Columbia, Kaslo, Salmon Arm, Duncan.

*Food Plant. Populus balsamifera,* according to Bobcaygeon rearings; apple, according to data on Kaslo specimen.

### *Peronea caliginosana* Wlk.

Page 325, fig. 7; Page 329, fig. 4.

*Teras caliginosana* Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 309. 1863.

This perfectly good species has been heretofore confused with *hastiana,* following an erroneous reference by Walsingham; a tentative identification, made after a study by the writer of a photograph of the type, was confirmed by Mr. W. H. T. Tams of the British Museum to whom specimens for comparison with this type were sent.

The species is one of our largest *Peroneas* and the decidedly mottled nature of the hindwings, combined with the rather even deep purple or gray-brown color of the forewings and an obsolescent maculation renders it easily recognizable; the few western specimens examined show a tendency toward a paler, more grayish, ground-color. The type of male genitalia is distinctly that of the *latifasciana* group.

*Genitalia, Male* (Three slides examined). Tegumen without cristae; anal sheath unarmed; socii *large, erect*, broad, reaching beyond apex of tegumen; sacculus with prominent truncate projection from lower margin apically, preceded by a deep, narrow emargination, hair-tuft short, broad; aedeagus moderately long and wide, vesica armed with a bundle of 8–10 short cornuti.

*Genitalia, Female* (Two specimens examined). Ostium plate with long, narrow, lateral lobes, pointed apically; ostium broad, sinuate, situated near cephalic margin of plate; chitinized portion of ductus bursae, long, broad, extending as far as the ends of the lateral lobes; membranous portion of ductus broad, narrowed somewhat at half, the inception of the ductus seminalis near bursa which is rather small and armed with a large, wedge-shaped, spined signum.

*Distribution.* Nova Scotia, Digby; Quebec, Meach Lake; Ontario, Ottawa; Alberta, Edmonton; British Columbia, Kaslo, Salmon Arm, Vancouver, Victoria.

*Food Plant.* Unknown.

### *Peronea latifasciana* Haw.

Page 325, fig. 5; Page 329, fig. 7.

*Tortrix latifasciana* Haworth, Lepid. Brit. 414. 1811.
*Acalla schalleriana* Kennel (*nec* Linn.), Palaearct. Tort. 90, Pl. V, figs. 31-34. 1909.
*Argyrotoxa schalleriana* Pierce and Metcalfe (*nec* Linn.), Genit. Brit. Tort. 17, Pl. VII. 1922.
*Phylacophora schalleriana* Filipjev, Ann. Mus. Zool. Acad Sci. U. R. S. S. 508. 1930.
*Peronea latifasciana* Sheldon, Entomol. LXIV, 30. 1931.

This species has been generally known as *schalleriana* Linn., but Sheldon has shown that the latter name must be applied to the species heretofore known as *logiana*, which leaves the name *latifasciana* available for the species under discussion.

In North America the species occurs in both the typical form, *i.e.*, with broad pale oblique band across the entire forewing and ruddy-brown outer area (Kennel, fig. 32), and the form *labeculana* Freyer (Kennel, fig. 31) in which only a large costo-apical triangle of either a red-brown or black-brown color is present. The male genitalia fit in excellently with Pierce's figure (as *schalleriana*), showing the faint spiculation of the anal sheath and the same shape of the sacculus emargination; in the female the shape of the lateral lobes of the ostium plate shows a slight difference from Pierce's figure in all three specimens examined, the bases being abruptly broadened, but this feature may occur in British specimens when sufficient material is available for study.

It should be noted that the species going under the above name, which is so frequently intercepted in the larval or pupal state on azaleas imported from Holland and Belgium, appears to show constant, slight genitalic differences which may indicate specific or at least racial distinctness. The genitalia agree remarkably well with Pierce's figures of *comparana* Hbn.; in the male the anal sheath lacks spiculation, the sacculus emargination is rectangular and the aedeagus is distinctly smaller and without any cornuti (Pierce's text states "5 small and 1 larger cornuti," but the figure does not show them). Until our European workers are able to clear up this apparent confusion the azalea pest may be known as *latifasciana* var. *comparana* Hbn. The male aedeagus figured by Filipjev (*op. cit.* Pl. XXV, fig. 2) does not fit in with any of the slides examined nor with Pierce's figures and is apparently that of some other species.

*Genitalia, Male* (Three slides examined). Tegumen broad; anal sheath with a distinct ventro-apical spine and finely spiculate; socii very large, erect, extending beyond apex of anal sheath; sacculus narrowly emarginate beyond half, the outer edge of emargination produced downward to form a fine spine; aedeagus very short, broad and only slightly bent, vesica armed with six to eight small apical spines arranged in two groups.

*Genitalia, Female* (Three slides examined). Ostium plate with moderately long, parallel lobes, broadening suddenly at base; ostium broad, almost straight, situated on cephalic margin of ostium plate; ductus bursae rather short and entirely membranous, enlarged slightly near base and containing a lightly chitinized plate; signum absent.

*Distribution.* Quebec, Meach Lake; Ontario, Ottawa, Trenton; Alberta, Edmonton.

*Food Plants.* *Salix, Vaccinium* (in Europe).

### ***Peronea schalleriana*** Linn.

Page 325, fig. 9; Page 329, fig. 9.

*P. Tortrix schalleriana* Linnaeus, Syst. Nat. X. 1758.
*Peronea viburnana* Clemens, Proc. Acad. Nat. Sci. Phil. 347. 1860; Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 485. 1924.
*Teras viburnana* Robinson, Trans. Am. Ent. Soc. II, 281, Pl. VII, fig. 66. 1869.
*Teras tristana* var. *famula* Zeller, Verh. Zool. Bot. Gesell. Wien, XXV, 214. 1875.
*Acalla logiana* Kennel (*nec* Linn.), Palaearct. Tort. 77, Pl. IV, figs. 34-38. 1909.
*Eclectis logiana* Pierce and Metcalfe (*nec* Linn.), Genit. Brit. Tort. 18, Pl. VII. 1922.
*Peronea schalleriana* Sheldon, Entomol. LXIII, 195. 1930.

The unfortunate confusion in the nomenclature of this species has been satisfactorily cleared up by Sheldon and henceforth the *Viburnum*-feeder must be known under the name of *schalleriana* Linn. The names *viburnana* Clem. and *famula* Zell. have been used for our North American forms, but the writer cannot see that these differ in any marked respect from the European forms for which there are already prior names available; the range of variation is excellently shown in Kennel's figures.

*Genitalia, Male* (Two slides examined). Tegumen narrowed apically; anal sheath truncate apically, unarmed; socii long, erect, projecting beyond

end of tegumen; sacculus with a projecting arm at half; aedeagus rather small, tapering, vesica armed with a bundle of 15–20 small cornuti.

*Genitalia, Female* (One slide examined). Ostium plate with long, narrow, lateral lobes, slightly incurved apically; ostium simple, narrow, situated just above cephalic margin of plate; ductus bursae entirely membranous, rather short and narrow, of even width throughout, inception of ductus seminalis near proximal end; signum a narrow piece of scobinate chitin.

*Distribution.* Ontario, Bobcaygeon, Pt. Pelee; British Columbia, Salmon Arm.

*Food Plant. Viburnum.*

### ***Peronea oxycoccana*** Pack.

Page 325, fig. 8; Page 329, fig. 8.

*Tortrix oxycoccana* Packard, Guide Study Ins. 334. 1869; *id.*, 17th Ann. Rep. Sec. Mass. Brd. Agric. 239. 1870.

As noted in the original description, the forewings of the species have a smooth, shiny look which should be sufficient to separate it from some of the dark forms of *minuta* Rob. which are superficially somewhat similar. The species is quite variable in coloration of forewings; in a majority of specimens these are evenly deep black-brown with scattered ruddy scaling; at times, however, they may be rather bright red-brown and specimens occur in which the predominant scaling is light gray. In the paler specimens a ruddy brown, broken, costal, triangular patch is distinctly visible. The genitalia are remarkably close to those of the preceding species.

*Genitalia, Male* (Four slides examined). Very similar to those of *schalleriana*, with the same erect socii and a sacculus arm at half which, however, is slightly narrower and more pointed; aedeagus longer and more attenuated apically, vesica with a slightly different arrangement of cornuti, which consist of two longer ones and a bundle of 8–10 short ones.

*Genitalia, Female* (Three slides examined). Similar to *schalleriana*, but ostium plate higher, more nearly square, with shorter lateral lobes; ostium much as in *schalleriana;* ductus bursae very short and faintly chitinized throughout with inception of ductus seminalis nearer the bursa; signum as in *schalleriana.*

*Distribution.* Ontario, Constance Bay (near Ottawa); Manitoba, Aweme; Alberta, Edmonton.

*Food Plant.* Cranberry and probably other *Vaccinium* species.

### ***Peronea variana*** Fern.

Page 325, fig. 10; Page 329, fig. 10.

*Teras variana* Fernald, 5th Rep. U.S. Ent. Comm. 847. 1890.
*Teras angusana* Fernald, Can. Ent. XXIV, 178. 1892.

This well known economic pest, the larva of which is known as the black-headed bud-worm, has caused considerable damage at various times to our Canadian forests of spruce, balsam and hemlock, notably in Cape Breton Island and British Columbia.

The moth well deserves its name as there seems no end to the countless variations of color and pattern it may assume; in this respect it quite equals

the European *hastiana* and its variations occur along similar lines. The scope of the present paper does not permit of a detailed discussion of the variation, but it might be well to note that while the superficially very dissimilar *angusana* is placed in the synonymy of the species on account of the practical similarity of genitalia, this particular variation occurs apparently only very rarely among the adults from spruce- and balsam-feeding larvae; on the other hand, a long series in our collections, showing only a moderate amount of variability, was beaten from cedar at Lyn, Ontario; the reddish-brown ground color (paling at times to a yellow-brown) was present in all specimens and the main variability occurred in the distinctness of the streak from base to apex and its bordering white shade. There is the possibility that *angusana* is an incipient species, the larva of which has become restricted to cedar as a food-plant.

The genitalia combine characters present in several distinct groups of *Peronea* and it is quite possible that the species is a comparatively primitive one.

*Genitalia, Male* (Three slides examined). Tegumen with the terminal lobes widely separated, with indications of cristae; anal sheath *bifid* at tip, a character only found in *hudsoniana;* the lower lateral points of the sheath are united by a thickened membrane to the sides of the tegumen, the whole forming a hood-like structure; socii *drooping*, produced distally; sacculus with ventral margin produced apically to a sharp spine preceded by a narrow emargination as in the *latifasciana* (*schalleriana* Auct.) group; aedeagus short and stout, as in the same group; vesica with a single short spine.

*Genitalia, Female* (Two slides examined). Very distinct. Ostium plate with long, narrow lobes, wide apart, each terminating in a sharp spine, inwardly directed; the caudal region of this plate (upper, in figure) is more heavily chitinized and strongly excavated in median section, forming a species of flap which seems to protect the terminal end of the long, narrow, flask-shaped ostium; the other end of the ostium protrudes considerably between the lateral lobes of the ostium plate; ductus bursae entirely membranous, broadly distended at its inception, narrowed greatly at half its length; bursa very small, without signum.

*Distribution.* Across the entire Dominion of Canada, following the spruce belt; typical *angusana* only known from Ottawa region and Lyn, Ontario.

*Food Plants.* White spruce, balsam, hemlock; *angusana* probably on cedar.

### *Peronea chalybeana* Fern.

Page 326, fig. 1; Page 330, fig. 1.

*Teras chalybeana* Fernald, Trans. Am. Ent. Soc. X, 65. 1882.
*Peronea chalybeana* Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 484. 1924.

A rather large, chunky species with olive-gray primaries and essentially the *hastiana* type of maculation. As noted by Forbes, specimens frequently occur with a blackish basal patch; in others, with obsolescent maculation and paler ground color, the costal triangular patch, with its partial border of black scales, is the most prominent feature of the maculation. In the male

genitalia, the type of socii appears to lead over to the *latifasciana* group, although other characters do not bear this out.

*Genitalia, Male* (Two slides examined). Tegumen broad, without cristae; anal sheath projecting strongly beyond apex of tegumen, unarmed ventrally; socii large, erect, broad at base, tapering apically; sacculus with ventral margin gently sinuate, apical hair-tuft short, broad; aedeagus long, thin, bent strongly; vesica armed with a small rod-like piece of chitin with small apical spine, and three long, fine cornuti, one subapical and the other two close together in central portion.

*Genitalia, Female* (Two slides examined). Ostium plate narrowly rectangular with short, broad, lateral lobes; ostium broad, slightly concave; chitinous portion of ductus bursae very long, tubular; membranous portion of ductus distended proximally, strongly narrowed beyond the inception of the ductus seminalis; signum an irregularly shaped piece of chitin with strong spines.

*Distribution.* Quebec, Chelsea, Meach Lake, Kazabazua; Ontario, Ottawa.

*Food Plant.* Unknown.

### *Peronea senescens* Zell.

Page 326, fig. 2; Page 330, fig. 2.

*Teras senescens* Zeller, Verh. Zool. Bot. Ges. Wien, XXIV, 431. 1874.

The species has been sunk in the synonymy of *nigrolinea* Rob. from which it is abundantly distinct on genitalic characters. It is slightly smaller in size and narrower winged than *nigrolinea* with less evident raised scale-tufts and a quite variable pattern on forewings. The ground color is light to smoky gray, in some specimens without much maculation except a light sprinkling of black dots; in others the whole median area is strongly suffused with deep smoky; in others again there are more or less distinct oblique sub-basal and median bands of a smokier gray than the ground color; a rarer form shows a black streak from base to apex of wing. The general type of genitalia is that of the *hastiana* group.

*Genitalia, Male* (Three slides examined). Tegumen broad, without cristae. Anal sheath with a short stout spine with broad base ventro-apically; socii roughly circular; sacculus strongly sinuate, the apical hair-tuft small and subrectangular; aedeagus rather short and stout, vesica armed with a single strong spine (rose thorn) and a minute cornutus.

*Genitalia, Female* (Two slides examined). Ostium plate narrow, with long, lateral lobes, directed outward and very wide apart, pointed apically; ostium broad, concave, situated at cephalic margin of plate; chitinized portion of ductus bursae very short; membranous portion of ductus rather evenly narrow with slight bulbous enlargement proximally just below which is the inception of the ductus seminalis; bursa contracted somewhat before fundus, with a large stellate signum.

*Distribution.* British Columbia, Victoria (Saanich District), probably extending into the western United States.

*Food Plant.* Unknown.

## *Peronea hastiana* Linn.

Page 326, fig. 3.

*P. Tortrix hastiana* Linn., Syst. Nat. X, 532. 1758.
*Acalla hastiana* Kennel, Die Palaearct. Tort. 20, Pl. III, figs. 21-43. 1908.
*Eclectis hastiana* Pierce and Metcalfe, Gen. Brit. Tort. 18, Pl. VII, (♀), Pl. VIII, (♂, as *maccana*). 1922.
*Peronea hastiana* Sheldon, Entomol. LVI, 75, 1923; *id.*, *op. cit.* LXIII, 148. 1930.
*Peronea hastiana* Forbes, Mem. 68, Cornell Univ. Agr. Expt. Sta. 485 (*partim*). 1924.
*Peronea hastiana* Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S., 507, Pl. XLI, fig. 1. 1929; *id.*, *op. cit.* 516, Pl. XXIII, fig. 3, Pl. XXVIII, fig. 6. 1930.

Probably no other species of *Peronea* has been so consistently misidentified in North America as *hastiana*. This is largely due to the known variability of the species in Europe and the consequent attempt on this continent to place a number of good species, which superficially approach *hastiana* forms in color and maculation, under this name; Forbes, for instance, following Dyar's catalogue, places no less than six names under *hastiana*, none of which, according to genitalia, should belong here. In consequence of this confusion it is impossible to recognize the true records of *hastiana* in North America from the misidentifications, and reference to this literature has therefore been omitted.

Even in Europe the species has been subject to misidentification and confusion, and it is only as recently as 1929 that accurate figures of the male and female genitalia have been presented by Filipjev. Several slides made from European material agree with his figures excellently and based on a comparison with these the true *hastiana* has been found to occur fairly commonly in our western provinces. In the specimens examined the variability of pattern is considerable; apart from the more or less typical form, *i.e.*, specimens with deep ruddy brown forewings crossed by an oblique antemedian whitish band, a form with the band of a reddish color is predominant, whilst more unicolorous specimens occur in which the band is almost entirely lost.

*Genitalia, Male* (10 slides examined). Tegumen with two distinct apical projections (cristae of Filipjev); anal sheath with a small ventro-apical spine; socii moderately large, drooping and roughly triangular; sacculus strongly emarginate at half with rugose edge beyond emargination, apical hair-tuft moderate, rather narrow; aedeagus short, stout, curved, vesica armed with a small, triangular, chitinous plate and four (very occasionally three) fine spines.

*Genitalia, Female* (Five slides examined). Ostium plate with long, broad, lateral lobes; ostium with raised chitinous lateral flaps; ductus bursae broad, strongly chitinized proximally to a point beyond the termination of the lobes, with a rounded lateral projection on each side; membranous portion of ductus rectangularly broadened proximally, narrowed considerably beyond half; signum small, stellate.

The length and width of the chitinized portion of the ductus appear to be somewhat variable and on the whole the female genitalia offer less reliable characters than do the male organs whereby the species may be differentiated from the two following ones.

*Distribution.* Manitoba, Aweme; Saskatchewan, Indian Head; Alberta, Edmonton, Red Deer, Fish Creek; British Columbia, Salmon Arm.

*Food Plant. Salix* (in Europe). No food plant records definitely known from North America.

### *Peronea celiana* Rob.

Page 326, fig. 4; Page 330, fig. 3.

*Teras celiana* Robinson, Trans. Am. Ent. Soc. II, 283, Pl. VII, fig. 74. 1869.
*Acleris albilineana* Kearfott, Can. Ent. XXXIX, 158. 1907.

Robinson's figure of this species is not at all bad. The coloration of the forewings in normal specimens is distinctly red-brown and there is an oblique whitish antemedian band, sprinkled with dark dots, and with rather waved edges. The thorax shows distinct red-brown tufting, which apparently is lacking in *hastiana*. *Albilineana* Kft. of which a Paratype is in the Canadian National Collection, proves to be a form of *celiana*, analogous to one of the European forms of *hastiana*. Both forms (in the dozen or so specimens examined) are remarkably constant and show neither intergrades nor variations.

*Genitalia, Male* (Four slides examined, one being of *albilineana*). Abundantly distinct from *hastiana*. Cristae of tegumen larger and closer together; ventro-apical armature of anal sheath consists of a transverse ridge of chitin rather than a pointed spine; socii long and narrow, the distal ends being much more drawn out than in *hastiana;* ventral margin of sacculus more sinuate beyond the median emargination than in *hastiana* and with the apical hair-tuft shorter and broader at base; armature of vesica consists of a strong spine situated on a heart-shaped chitinous base and a bundle of short, fine spines, apparently variable in number (6–12).

*Genitalia, Female* (Two slides examined, one being of *albilineana*). Considerably larger than in the preceding species, but essentially of same type; lateral projections of the chitinous portion of the ductus much larger and the lateral lobes of the ostium plate somewhat more pointed than in *hastiana*.

*Distribution.* Quebec, Knowlton, Aylmer; Ontario, Ottawa, Biscotasing; Alberta, Edmonton.

*Food Plant. Betula*, according to specimen bred by the writer at Knowlton, Quebec.

### *Peronea pulverosana* Wlk.

Page 326, fig. 5; Page 330, fig. 4.

*Teras pulverosana* Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 291. 1863.

There is still some doubt as to whether this name is correctly applied; the type male in the British Museum from St. Martin's Falls, Albany river, Ontario, is without abdomen and, in consequence, it has been impossible to examine the genitalia. However, a photograph of this type is before the writer and Mr. W. H. T. Tams of the above institution has kindly picked out and labelled as agreeing with the type one of a number of specimens sent for comparison after a study of the photograph; the determination rests for the present on this specimen.

The species is very close to *hastiana* and would appear, as far as present knowledge goes, to represent it in the eastern portion of Canada. The maculation is essentially as in *hastiana;* in most of the specimens there is less of the ruddy shade in the ground color, and beyond the oblique band the fore-

wing in several of the males appears to be more splotched with a paler gray color, similar to that of the band; two of the males show a distinct dark streak extending from base of wing along the cubital vein to end of cell. The females are smaller and duller in coloration.

*Genitalia, Male* (Five slides examined). Quite similar to those of *hastiana*, but differing in all specimens examined in the following points; the socii are less drawn out distally and consequently more nearly circular in outline; the aedeagus is slightly longer and narrower and the armature of the vesica consists, besides the triangular piece of chitin, of only *three* spines, somewhat finer and shorter than those of *hastiana;* generally the proximal edge of the excavation of the sacculus forms more nearly a right angle with the basal portion, but this is probably a variable character.

*Genitalia, Female* (Two slides examined). Quite similar to those of *hastiana* and cannot be satisfactorily differentiated at the present time.

*Distribution.* New Brunswick, Fredericton (1 ♂); Quebec, Hull (1 ♂), Meach Lake (1 ♀); Manitoba, Aweme (3 ♂, 1 ♀).

*Food Plant.* Unknown.

### *Peronea walkerana* n. sp.

Page 326, fig. 6; Page 330, fig. 5.

*Male*

Size of *hastiana*. Head, thorax and forewings a very dark smoky-gray with scarcely a trace of maculation. The oblique antemedian band is very faintly indicated by a few dark scales near costa representing its outer margin and there are scattered blackish scales in apical portion (in one specimen intermingled with stray brown scales). Secondaries pale smoky, slightly speckled with black near apex of wing.

*Female*

Very similar to male; raised blackish scales on inner and outer margins of the antemedian band better defined than in male, with traces of the deeper costal shading beyond the band usual in members of this group.

*Genitalia, Male* (Two slides examined). Tegumen with cristae present, but reduced; spine of anal sheath stronger than in *pulverosana;* socii much as in *pulverosana;* sacculus sharply, rectangularly emarginate before half, then almost straight to apical tuft; aedeagus short, stout, vesica armed with an oblong piece of chitin with a raised ridge and *four* spines, considerably *chunkier and shorter* than in *hastiana*.

*Genitalia, Female* (Three slides examined). Abundantly distinct from *pulverosana*. Lateral lobes of ostium plate shorter and broader; ostium broad, concave, *without lateral flaps;* proximal portion of ductus bursae well chitinized and extending beyond lateral lobes but *without lateral projections;* membranous portion of ductus enlarged proximally, bulb-shaped, containing a large, more or less dumb-bell shaped, lightly chitinized plate; whole ductus shorter than in *pulverosana;* signum stellate.

*Holotype* ♂, Trenton, Ontario, April 21 (J. Evans); No. 3745 in the Canadian National Collection, Ottawa.

*Allotype* ♀, Aylmer, Quebec, April 28 (C. H. Curran); in same collection.

*Paratypes* 1 ♂, Merivale, Ontario, April 16 (G. S. Walley); 1 ♀, Aylmer, Quebec, April 25 (C. H. Curran); 1 ♀, Trenton, Ontario, June 28 (J. Evans).

The type specimens are evidently hibernated ones and it is probable that in fresh material the maculation will be better defined. The species should not, however, be difficult of recognition, especially in the female sex, after an examination of the genitalia.

*Food Plant.* Unknown.

### ***Peronea robinsoniana*** Forbes

Page 326, fig. 7; Page 330, fig. 6.

*Teras flavivittana* Robinson (*nec* Clemens), Trans. Am. Ent. Soc. II, Pl. VII, fig. 61. 1869.
*Peronea robinsoniana* (Kft.), Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta., 487. 1924.
*Peronea clemensiana* (Kft.), Forbes, *op. cit.* 487. 1924.

The above two names of Kearfott were validated by Forbes in his "Lepidoptera of New York and Neighboring States." The writer has examined, through the courtesy of the American Museum authorities, a series of both forms from the Kearfott collection which he believes may be regarded as co-types and has so marked them. *Robinsoniana* appears to be what Robinson figured under the name *flavivittana* Clem. and *clemensiana* is only a darker form of the same, the forewings being largely suffused with black-brown. Unfortunately neither of the names represents the normal form which is so close in color and maculation to *inana* Rob. as to be almost indistinguishable without a reference to the genitalia. The size is slightly smaller than in *inana* and the coloration of the forewings rather brighter; there is, however, the same whitish basal area defined outwardly by an oblique line and containing a large triangular dark patch on inner margin; the outer half of wing is red-brown with at times slight darker suffusions in costal area. The species is a very variable one, the pale basal area being frequently suffused with the same ruddy-brown found in the apical area; other specimens show a more or less even red-brown shade over the entire wing with white streaks, tending to form a band, along the inner margin, and slight streaking of a similar color below the apex of the wing.

*Genitalia, Male* (Eight slides examined). Cristae of tegumen and spine of anal sheath much as in *hastiana;* socii narrower and more nearly triangular than in *hastiana*, with the distal portion considerably more produced at both ends; sacculus emarginate at half, edge rugose beyond emargination with a *small, but distinct pointed flap* of chitin; aedeagus short, chunky, vesica armed with a short stout spine (rose-thorn of Pierce) and *five* longer, thinner spines arranged in two groups.

*Genitalia, Female* (Five slides examined). Ostium plate narrower than in *hastiana;* ostium without the lateral flaps, openly V-shaped; chitinized portion of ductus without lateral projections. Otherwise much as in *hastiana*.

*Distribution.* Manitoba, Aweme; Alberta, Edmonton; British Columbia, Salmon Arm, Vancouver. Also occurring at Hampton, New Hampshire, and Winchendon, Massachusetts.

*Food Plant.* Not definitely recorded, possibly *Vaccinium.*

### *Peronea flavivittana* Clem.

Page 326, fig. 8; Page 330, fig. 7.

*Peronea flavivittana* Clemens, Proc. Ent. Soc. Phil. III, 516. 1864.
*Teras perspicuana* Robinson, Trans. Am. Ent. Soc. II, 280, Pl. VII, fig. 62. 1869.

The so-called "type" specimen of this species in the collection of the Academy of Natural Sciences, Philadelphia, will have to be rejected as such, according to notes recently made for the writer by Mr. W. J. Brown. This spurious type fits in well with Robinson's figure of the species, Plate VII, fig. 61, but contradicts entirely the original description, the yellow stripe along the inner margin, on which the name was based, being entirely lacking. However, a specimen from New Brighton, Pennsylvania, is before the writer which agrees excellently with Clemens' diagnosis and the identity of the species is based on this specimen.

The typical form is evidently an aberrant one of rather rare occurrence; the more normal form is the one which has been distributed by collectors in Pittsburgh, Pennsylvania, and vicinity under the name *hastiana* and has the forewings of a general blackish-brown color with light brown spotting along costa and outer margin and strong scale-tufting which give the wings a rather roughened appearance. There is considerable variation in the series examined, paler specimens showing a deep blackish basal area and a more or less evident oblique antemedian band varying in color from light creamy to pale brown; the terminal area being purple-brown; the name *perspicuana* Rob. is probably based on an extremely pale specimen of this nature; the locality "Pennsylvania" for the type specimen would certainly point in this direction. Other specimens are more evenly purple-brown with pale streaking along inner margin, quite reminiscent of some of the forms of *robinsoniana*, and others again show heavy black longitudinal streaks through the centre of wing between base and apex.

*Genitalia, Male* (Seven slides examined). Cristae of tegumen well developed; anal sheath *without* ventro-apical spine; socii smaller and less produced distally than in *hastiana;* sacculus with deep emargination at half, edge strongly rugose beyond; aedeagus *thin, long,* strongly bent, *armed with an apical spine;* vesica with two stout cornuti, situated one above the other.

*Genitalia, Female* (Five slides examined). Ostium plate with long lateral lobes; ostium rather narrow, concave, with strong, lateral, spiculate flaps; chitinous portion of ductus bursae short, not extending as far as ends of lateral lobes, the following membranous portion somewhat distended to form a long, oval sack, very faintly chitinized and longitudinally streaked; beyond this the ductus is long and narrow with the ductus seminalis situated about midway between the end of the basal sack and the bursa; signum large, stellate.

*Distribution.* Ontario, Ottawa (this record based on a single female). Other records are Hampton, New Hampshire; Clinton, New York; Pittsburgh and Oak Station, Pennsylvania.

*Food Plant.* Unknown.

### *Peronea youngana* n. sp.

Page 326, fig. 9; Page 330, fig. 10.

Very evidently allied to the preceding species, but somewhat smaller and paler. Palpi, head, and thorax light gray-brown; primaries light gray-brown with scattered black sprinkling; a strong black dash extends from base of wing along cubital vein to end of cell; a second black dash, starting immediately costad of the end of the first one, continues to apex of wing, both dashes bordered with red-brown scaling; a strong oblique row of raised scales extends across median area of wing and there is a scale tuft on each side of the black basal dash at slightly less than half its length, the lower tuft much the stronger. Secondaries pale smoky, deeper outwardly. Expanse, 18 mm.

In one male paratype the color of primaries is rather even deep gray and in place of the black dashes there is a broad burnt-orange fascia from base half-way to apex; in a female paratype the black dashes are lacking.

*Genitalia, Male* (Two slides examined). Cristae of tegumen strongly developed; ventro-apical spine of anal sheath *long and spatulate* at tip; socii broad and roughly equilaterally triangular, much larger than in *flavivittana;* sacculus *drawn out to a sharp point* at half, followed by the usual emargination; aedeagus thin, somewhat tapering, armed with a short apical spine; vesica with an apical bundle of short spines, approximately six to eight in number.

*Genitalia, Female* (Two slides examined). Ostium plate with long, broad lobes; ostium rather narrow, strongly convex, with smaller lateral flaps than in *flavivittana;* proximal chitinous portion of ductus bursae short, scarcely extending below lower margin of ostium plate, followed by a bulbous membranous dilation which in turn narrows to form a thin tube; the proximal end of this, almost as far as the inception of the ductus seminalis, is lightly chitinized. Signum moderate, stellate.

*Holotype* ♂, Ottawa, Ontario, June 16, 1905 (C. H. Young); No. 3747 in the Canadian National Collection.

*Allotype* ♀, same locality and collector, September 25, 1905, in same collection.

*Paratypes* 1 ♂, 2 ♀, same data, September 3, 16; October 1, 1905.

*Food Plant.* Unknown.

### *Peronea britannia* Kft.

Page 326, fig. 10; Page 330, fig. 9.

*Acleris britannia* Kearfott, Can. Ent. XXXVI, 138. 1904.

An easily recognized species with pale orange-yellow forewings with large purple-brown pale-centered costal triangle and slight dark sprinkling or reticulation; the variation in the series examined is very slight. On Vancouver Island the larvae are reported as injurious to leaves of young loganberry canes. According to genitalic characters the species would fall in the *hastiana* group.

*Genitalia, Male* (One slide examined). Tegumen with distinct cristae; anal sheath with small ventro-apical spine; socii subtriangular, decumbent, produced distally; sacculus with emargination at half; aedeagus rather short, chunky, *armed with sharp subapical spine*, vesica with two pairs of moderately long cornuti.

*Genitalia, Female* (One slide examined). Ostium plate narrow, with broad lateral lobes situated wide apart; ostium broad, slightly convex; ductus bursae with proximal section flask-shaped, strongly chitinized to a point beyond ends of lateral lobes; membranous portion of ductus broadened proximally as in *hastiana*, considerably narrowed at half, beyond which is the inception of the ductus seminalis; signum large, stellate.

*Distribution.* Alberta, Banff; British Columbia, Revelstoke, Kaslo, Salmon Arm, Victoria, Departure Bay.

*Food Plants.* Loganberry, rose.

## *Peronea fragariana* Kft.

Page 326, fig. 11; Page 330, fig. 8.

*Acleris fragariana* Kearfott, Can. Ent. XXXVI, 140. 1904.
*Peronea fragariana* Criddle, 60th Ann. Rep. Ent. Soc. Ont., 25. 1929.

The species has been confused with the European *permutana* Dup. from which it is distinct on genitalic characters; it is to quite an extent a pest on strawberry plants in the western provinces where it is known as the strawberry leaf cutter. The forewings are normally pale orange-yellow in the basal half with reddish-purple suffusion over the outer area and broadly along inner margin to base. The only eastern specimens examined are entirely suffused with deep purple-brown shades on the forewings. On genitalic characters the species shows points of contact with the *inana* group.

*Genitalia, Male* (One slide examined). Tegumen strongly produced, with slight traces of cristae; anal sheath armed with a blunt ventro-apical spine; socii large, subtriangular, well-produced at both ends; sacculus with lower margin straight, except for a small semicircular projection with rugose edge at half; aedeagus moderately long and narrow; vesica with a strong curved apical spine and three minute cornuti.

*Genitalia, Female* (One slide examined). Ostium plate large with convex caudal margin and broad lateral lobes, rather close together; ostium near cephalic edge of plate, straight, broad; ductus bursae with the proximal chitinous portion long, tubular, extending well beyond apex of lateral lobes; membranous portion broadened proximally much as in *hastiana*, then considerably narrowed with inception of ductus seminalis near distal end; bursa large with large stellate signum.

*Distribution.* New Brunswick, Fredericton; Manitoba, Aweme, Souris; Saskatchewan, Saskatoon.

*Food Plant.* Strawberry and probably other allied plants.

### *Peronea inana* Rob.

Page 327, fig. 1; Page 331, fig. 1.

*Teras inana* Robinson, Trans. Am. Ent. Soc. II, 281, Pl. VII, fig. 65. 1869.

The determination of this species is based on a specimen compared by the writer in 1925 with a series in the United States National Museum under this name, and by a recent examination of a specimen in the American Museum Collection labelled "Homotype" by Kearfott. The small series of 2 ♂, 4 ♀, in the Canadian National Collection shows no sign of variation and agrees quite well with Robinson's figure, but is disconcertingly close in general appearance to what has been already discussed as the normal form of *robinsoniana*. As far as can be told, *inana* is a rather larger species, with less prominent pure white scaling on rear portion of thorax and with the basal area of forewing rather evenly dull gray-brown without the contrasting white scaling or the obscuring blackish shading frequently found in *robinsoniana* forms. Until, however, one knows the species well, the genitalia form the only safe means of determination.

*Genitalia, Male* (Two slides examined). Tegumen *without* cristae; no armature to anal sheath; socii long, well-produced distally, suboval in shape; sacculus *without* emargination, but with *small semicircular projection* beyond half; aedeagus short, moderately stout, vesica armed with a strong, short, subapical spine (rose-thorn) and a bundle of about a dozen small cornuti.

*Genitalia, Female* (Four slides examined). Ostium plate rather narrow from front to rear, lateral lobes broad, long and rather wide apart; ostium broad, concave; ductus bursae broad proximally and strongly chitinized for a considerable length to a point beyond ends of lateral lobes, expanding somewhat laterally at termen; membranous portion of ductus as broad as chitinous portion at commencement, but narrowing rapidly, forming a long narrow tube beyond the inception of the ductus seminalis. Signum large, roughly circular, scobinate.

*Distribution.* Ontario, Ottawa; Alberta, Edmonton.

*Food Plant.* Unknown.

### *Peronea busckana* n. sp.

Page 327, fig. 2; Page 331, fig. 3.

*Female*

Palpi, head and thorax purple-gray, the latter shaded on patagia and tuft with brown and crossed by a median black line. Forewings light violet-gray shaded with deeper purple-gray. At base of wing a small black-brown patch, shaded slightly with light brown scaling; just beyond this in the fold a smaller dark patch with brownish scaling and a dark scale-tuft, this patch enclosed in a broad oblique band of the violet-gray ground color which appears broader at inner margin than at costa and is defined outwardly in costal half and on inner margin by an oblique row of 4 or 5 minute, blackish, slightly raised dots, faintly and partially surrounded with light brown scaling. Beyond this band, in costal half of wing a broadly triangular patch, extend-

ing nearly to apex of wing, is obscurely indicated by darker, purple-gray suffusion. The terminal area of wing is violet-gray with a few scattered black dots. Fringes light violet-gray with some darker scales at base. Secondaries very pale smoky, somewhat darker apically.

*Male*

Rather paler than the female in color with the basal patch and costal triangle less obvious; the slightly raised dark dots partially defining the outer edge (and at times the inner edge) of the antemedian band are, however, quite prominent and there is a further more or less incomplete line of similar dark dots from costa at about half to inner margin before anal angle, strongly excurved opposite cell. These dots at times give a very characteristic appearance to the wing. Expanse, ♂, 20–22 mm., ♀, 20 mm.

*Genitalia, Male* (One slide examined). Cristae of tegumen large and sharply pointed; anal sheath with moderate ventro-apical spine; socii small, not produced distally; claspers bent inward in apical half; sacculus with straight basal portion, then with strong emargination beyond half, roughly rectangular at anal angle with strong terminal hair-tuft; aedeagus rather chunky; vesica armed with a large spindle-shaped piece of chitin and a smaller spine without base.

*Genitalia, Female* (Three slides examined). Very characteristic and quite distinct from all others in the group. Ostium plate with the lobes broad, but rather short, slightly bent outward at extremity; ostium broad, convex, forming a hood-like, heavily chitinized plate, the chitinization extending downward along the inner edges of the lateral lobes; the proximal, strongly chitinized portion of the ductus bursae with parallel sides, extending well beyond the ends of lobes; membranous portion rather short, narrowed towards bursa. Signum roughly circular, scobinate.

*Holotype* ♀, Mer Bleue, near Ottawa, Ontario, September 18, 1902 (C. H. Young); No. 3748 in the Canadian National Collection, Ottawa.

*Allotype* ♂, Hampton, New Hampshire, October 10, 1907 (S. A. Shaw); in Collection of the American Museum of Natural History, New York.

*Paratypes* 1 ♀, Aweme, Manitoba, October 3, 1923 (N. Criddle); in Canadian National Collection; 6 ♂, 2 ♀, Hampton, New Hampshire, October 10, 16, 26, November 20, December 3, April 30, May 13; 4 ♂, 11 ♀, Winchendon, Massachusetts, October 12, 14, 20, 26, 28, 31, April 17, May 9, 25, in Collection of the American Museum of Natural History and Canadian National Collection.

A long series from the Kearfott Collection, loaned by the American Museum of Natural History, was very useful in augmenting the scanty number of specimens available for study in our own collections. There is very little variation in either color or pattern in the specimens examined.

This species somewhat resembles *similis* Filipjev in type of genitalia, especially in the female sex.

*Food Plant.* Unknown, but possibly *Vaccinium* or an allied plant.

### *Peronea maculidorsana* Clem.

Page 327, fig. 10; Page 331, fig. 2.

*Cnephasia ? maculidorsana* Clemens, Proc. Ent. Soc. Phil. III, 516. 1864.
*Teras maculidorsana* Robinson, Trans. Am. Ent. Soc. II, 281, Pl. VII, fig. 64. 1869.
*Peronea hypericana* Ely, Proc. Ent. Soc. Wash., XII, 68. 1910.

The species has probably been confused with both *inana* and *busckana;* it is a much grayer species than the former and Robinson's figures emphasize this difference quite clearly. From *busckana* it differs in having a dark lunate patch above inner margin near base of wing, which is entirely pale gray; this patch is much more clearly defined in the females than in the males which are frequently without much maculation or brown suffusion and superficially resemble closely *busckana*, the dark raised dots (when present) being similarly placed in both species; otherwise, there does not appear to be much variation. From the description and from memory of the type in the United States National Museum, *hypericana* Ely would appear to fall as a synonym.

*Genitalia, Male* (Two slides examined). Small for the size of the insect. Tegumen *without* cristae and anal sheath without armature; socii rather small, roughly triangular; sacculus margin almost straight with the exception of a small semicircular chitinous flap, projecting beyond half; aedeagus moderately stout, *armed with a short apical spine;* vesica with a bundle of small cornuti, variable in number (8–12 in specimens examined).

*Genitalia, Female* (Two slides examined). Ostium plate with *only an indication* of lateral lobes, ostium broad and close to lower margin of plate (as drawn); chitinized portion of ductus bursae short and roughly subrectangular; membranous portion broad, slightly enlarged proximally, bursa small with signum represented by a long, narrow, scobinate strip of chitin.

*Distribution.* Quebec, Aylmer, Kazubazua; Ontario, Constance Bay, Mer Bleue (both localities in the Ottawa region). Also occurs at Hampton, New Hampshire.

*Food Plant. Hypericum* (according to Ely). In the Ottawa region the normal food plant would appear to be *Vaccinium.*

### *Peronea scabrana* D. & S.

Page 327, fig. 3; Page 331, fig. 4.

*Tortrix scabrana* Denis and Schiffermuller, Syst. Verz. 130. 1776.
*Peronea scabrana* Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S. 507, Pl. XLI, figs. 2, 4. 1929.

This species, which has only recently been removed from the synonymy of *hastiana* by Filipjev, occurs also in North America. A single male in the Canadian National Collection from Calgary (*ex* Coll. Wolley-Dod) has been recently supplemented by several specimens secured by Mr. K. Bowman at Nordegg, Alberta.

As stated by Filipjev, the species is longer and narrower-winged than *hastiana* and appears to be as variable in color and maculation as this species. Our Calgary specimen has pale fawn-colored primaries with a blackish basal patch, the outer edge of which is acutely angled outwardly in middle of wing; the other specimens, although with similar genitalia, are of a more or less even deep gray color with very obscure maculation.

*Genitalia, Male* (Two slides examined). Tegumen broad and without cristae. Armature of anal sheath a long acute spine (longer than in European specimens); socii club-shaped, rugose rather than hairy; margin of sacculus gently sinuate; aedeagus long, narrow, with short terminal spine; vesica armed with a short, pointed piece of chitin and three cornuti.

*Genitalia, Female.* Ostium plate with long pointed lobes; ostium rather narrow, slightly concave; chitinized portion of ductus bursae short, subrectangular; membranous portion of ductus strongly enlarged in proximal half, much narrowed after inception of the ductus seminalis. Signum moderate, scobinate.

*Distribution.* Alberta, Calgary, Nordegg, Edmonton.

*Food Plant.* Unknown.

### *Peronea lipsiana* D. & S.

Page 327, fig. 9; Page 331, fig. 6.

*Tortrix lipsiana* Denis and Schiffermuller, Syst. Verz. 129. 1776.
*Acleris lipsiana* Kennel, Palaearct. Tort. 90, Pl. V, figs. 28-30. 1909.
*Peronea lipsiana* Pierce and Metcalfe, Gen. Brit. Tort. 22, Pl. IX (?). 1922.
*Peronea lipsiana* Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S., 516, Pl. XXIV, fig. 1; Pl. XXIX, fig. 4. 1930.
*Peronea lipsiana* Sheldon, Entomol. LXIII, 273. 1930.

The species has been heretofore unrecognized in North America and probably confused with *hudsoniana* Wlk. (*brewsteriana* Rob.). A small series from the western provinces, however, not only agrees excellently in maculation with Kennel's figures, but in genitalic characters matches exactly Filipjev's figures of these organs; Pierce's figures under this name show some discrepancies and only doubtfully represent the species.

The primaries of *lipsiana* are practically smooth, rather even dark gray with a slight fawn-colored tinge and sprinkled lightly with black dots; this sprinkling is particularly evident in the females, which are generally paler gray and considerably smaller in size. In another equally common form the ground color is ruddier with the costal triangle and a patch on inner margin near base indicated by smoky shading.

*Genitalia, Male* (Three slides examined). Tegumen narrow apically, without cristae; anal sheath unarmed; socii on a long, narrow, rod-like base, small, hemispherical, with short hairs; margin of sacculus gently arched beyond half, rugose; apical hair-tuft strong, pointed; aedeagus thin, vesica armed with a small triangular piece of chitin and a bundle of numerous long thin cornuti, about 15–18 in number.

*Genitalia, Female* (Three slides examined). Ostium plate with long, broad lobes, bent slightly inward apically; ostium broad, sinuate; ductus bursae with the chitinized portion unusually long and narrow, extending far beyond the ends of the lateral lobes; proximal end of membranous portion of ductus slightly enlarged and containing a chitinized plate below which is the inception of the ductus seminalis; remainder of ductus bursae short and rather broad; signum large, considerably longer than broad, well-spined.

*Distribution.* Manitoba, Aweme; Alberta, Edmonton.

*Food Plants. Vaccinium, Myrica* (in Europe).

## *Peronea hudsoniana* Wlk.

Page 327, fig. 8; Page 331, fig. 9.

*Teras hudsoniana* Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 290, (♂). 1863.
*Sciaphila implexana* Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 338, (♀). 1863; Walsingham, Ill. Lep. Het. Brit. Mus. IV, 3, Pl. LXI, fig. 7 (as *pulverosana* Wlk.). 1879.
*Teras brewsteriana* Robinson, Trans. Am. Ent. Soc. II, 283, Pl. VII, fig. 73. 1869.

The correct identity of the various specific names involved in the above synonymy has been difficult to determine. Walsingham's and Robinson's descriptions and figures distinctly indicated the sinking of *brewsteriana* to *implexana* and comparisons of specimens kindly made by Mr. W. H. T. Tams in the British Museum with the type female of *implexana*, and by Dr. E. T. Cresson, Jr., in the Academy of Natural Sciences, Philadelphia, with the type of *brewsteriana* confirmed this opinion. With regard to *hudsoniana*, however, Mr. Tams was unwilling to make a definite decision, the abdomen of the type being lost and the maculation of the specimen showing a marked similarity to that of several allied species. After a careful study of a photograph of the type, the writer has rightly or wrongly concluded that *hudsoniana* most probably was based on the male sex of *implexana;* in the fairly large series of the species available for study the males are consistently much larger than the females, more evenly deep gray in the coloration of the primaries, and with the brown shading beyond the discal dash, so characteristic for the female, much reduced or entirely lacking. There is little variation in our series except that occasionally in the male the forewings are irregularly splotched with small, dark patches.

The genitalia in both sexes are remarkably characteristic.

*Genitalia, Male* (Four slides examined). Tegumen without cristae; anal sheath *bifid* at apex, armed with a strong ventral spine; socii long, narrow, drawn to a point distally; sacculus strongly emarginate at half, abruptly and rectangularly produced costad at apex, which is raised above the plane of the clasper and tufted with hairs; aedeagus long, narrow, armed with a short terminal spine; vesica with three terminal cornuti, one of which is only half the length of the others.

*Genitalia, Female* (Five slides examined). Ostium plate *entirely without* lobes, ostium nearly straight, occupying the entire width of the cephalic (lower, in figure) margin of the plate; ductus bursae as broad as the ostium plate at its inception, strongly chitinized for a considerable length and asymmetrically narrowing; a large oval bulge from the under-surface occupies the adjacent portions of both the plate and the ductus; beyond the chitinized portion the ductus forms an irregularly shaped sack at the lower end of which the ductus seminalis has its inception; remainder of ductus narrow; bursa small with a minute stellate signum.

*Distribution.* Ontario, Ottawa; Manitoba, Aweme; Saskatchewan, Indian Head; Alberta, Fish Creek, Edmonton, Red Deer, Nordegg. Specimens also examined from Hampton, New Hampshire; Winchendon, Massachusetts; and Montclair, New Jersey.

*Food Plant.* Unknown.

### *Peronea bowmanana* n. sp.

Page 327, fig. 4; Page 331, fig. 5.

*Male*

Palpi deep purple-gray with slight ruddy scaling above; head and thorax deep purple-gray, latter with a few burnt-sienna scales on the tuft. Primaries almost unicolorous deep purple-gray (at times without purple shade) with a slight admixture of burnt-sienna scaling and a small ruddy shade at base of wing. A prominent tuft of creamy white raised scales (very easily rubbed off) surrounding two minute black scale-dots in the cell. Secondaries semi-hyaline, whitish, slightly smoky apically.

*Female*

Smaller than male; ruddy shades on thoracic tuft and at base of forewing more prominent; white discal scale-tuft very strong in unrubbed specimens. Secondaries somewhat smokier in color. Expanse, ♂, 22 mm., ♀, 18–20 mm.

*Genitalia, Male* (Four slides examined). Tegumen without cristae, broad, expanded laterally at bases of socii; anal sheath without armature; socii long, ribbon-like, pointed apically, shortly hairy in apical section; claspers short, chunky; sacculus slightly emarginate at half, then strongly rounded with short, broad apical tuft; apex of valvula thickened, rugose; aedeagus rather narrow, strongly bent, vesica armed with a single long stout spine.

*Genitalia, Female* (Two slides examined). Ostium plate with long, narrow lateral lobes, slightly directed inwardly; ostium broad, sinuate; chitinized portion of ductus bursae short, rectangular; membranous portion of ductus with a slight proximal enlargement, containing a faintly chitinized plate, this section slightly narrowed distally and followed by a further expansion to a short broad tube leading into the small bursa. Signum very minute, almost obsolete.

*Holotype* ♂, Edmonton, Alberta, October 1, 1932 (K. Bowman); No. 3750 in the Canadian National Collection, through the courtesy of the collector.

*Allotype* ♀, Aweme, Manitoba, October 3, 1923 (N. Criddle); in same collection.

*Paratypes* 1 ♂, 6 ♀, Edmonton, Alberta, March 28, April 22–29 (K. Bowman); in Canadian National Collection and collection of K. Bowman; 2 ♂, Aweme, Manitoba, October 2, May 13 (N. Criddle); in Canadian National Collection; 4 ♂, 1 ♀, Aweme, Manitoba, April 4, 8, 14 (Criddle) (ex Coll. Kearfott) in Collection of the American Museum of Natural History; 1 ♂, 2 ♀, Salmon Arm, British Columbia, October 17, March 13, May 7 (W. R. Buckell); in Canadian National Collection and collection of W. R. Buckell.

*Food Plant.* Unknown.

### *Peronea minuta* Rob.

Page 327, fig. 7; Page 331, fig. 8.

*Tortrix minuta* Robinson, Trans. Am. Ent. Soc. II, 276, Pl. VI, fig. 49. 1869.
*Tortrix vaccinivorana* Packard, 17th Ann. Rep. Sec. Mass. Brd. Agr. 241. 1870.
*Tortrix malivorana* Le Baron, 1st Ann. Rep. Ins. Ill. 20. 1870; Riley, 4th Ann. Rep. State Entom. Mo. 47. 1872.
*Tortrix cinderella* Riley, 4th Ann. Rep. State Entom. Mo. 46. 1872; *id.* Bull. 6, U.S. Entom. Comm. 82. 1881.
*Teras variolana* Zeller, Verhandl. zool. bot. Ges. Wien, XXV, 212. 1875.

This species is the well known pest on cranberry plants and the larva is known to economic entomologists as the yellow-headed fire worm. In the middle western States it was found destructive to apple trees and described as a new species, but Riley later concluded that apple and cranberry-feeders were identical and his views have been generally followed and are here accepted. The adults occur in two main color forms; the typical one with orange-yellow forewings, faintly reticulate with shining purplish scaling, occurs mostly in the spring and summer generations; the fall generation (*cinderella* Riley) which hibernates has the forewings deep smoky gray. In Canada the species is apparently not very common and is only found in bogs where blueberries abound. The characteristic genitalia show no very close relationship to any of the *Peronea* groups, but possibly link up best with the *hudsoniana-bowmanana* group.

*Genitalia, Male* (Six slides examined). Very small. Tegumen broad apically, especially at bases of socii; anal sheath weak, unarmed; socii long, narrow, bluntly pointed, with short scattered hair apically; claspers short; broad, sacculus strongly excavated beyond base, bent upward toward costa apically with raised hair-tuft; aedeagus large, broad; vesica armed with a chitinous plate with raised ridge and an apical cluster of 8 to 10 small cornuti.

*Genitalia, Female.* Ostium plate with very short lateral lobes; ostium broad, situated on cephalic margin of plate; chitinized proximal portion of ductus bursae long, broad, finely scobinate; membranous portion of ductus enlarged to an elongated sack shortly beyond base, narrowing at about half, where the inception of the ductus seminalis is situated; signum broadly oval, spined.

*Distribution.* Ontario, Ottawa (Mer Bleue); Manitoba, Aweme; Alberta, Fish Creek, Nordegg.

*Food Plants.* Apple, cranberry.

### *Peronea logiana* Linn.

Page 327, fig. 5; Page 331, fig. 7.

*P. Tortrix logiana* Linnaeus, Faun. Suec. (Ed. II), 348. 1761.
*Phalaena niveana* Fabricius, Ent. Syst. III (2), 267. 1794.
*Teras placidana* Robinson, Trans. Am. Ent. Soc. II, 282, Pl. VII, fig. 68. 1869.
*Teras trisignana* Robinson, Trans. Am. Ent. Soc. II, 282, Pl. VII, fig. 69. 1869.
*Acalla niveana* Kennel, Palaearct. Tort. 87, Pl. V, figs. 19, 20. 1909.
*Peronea niveana* Pierce and Metcalfe, Gen. Brit. Tort. 20, Pl. VIII. 1922.
*Peronea trisignana* Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 483. 1924.
*Oxigrapha logiana* Sheldon, Entomol. LXIII, 195. 1930; *id., op. cit.* LXIV, 101. 1931.
*Peronea niveana* Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S. 522, Pl. XXV, figs. 7, 8, Pl. XXIX, fig. 1. 1930.

The involved synonymy of this species in Europe has been satisfactorily cleared up by Sheldon; there seems no doubt that Robinson's two names represent North American forms of this same species, *placidana* falling to the

type form and *trisignana* taking priority (if a name be desired) over *tripunctana* Sheldon (*op. cit.* 103). Both Pierce and Filipjev state that in the male genitalia the vesica is armed with *three* spines; in the only European specimen examined *four* spines were present, which agrees with the number found in North American specimens.

*Genitalia, Male* (Three slides examined). Tegumen rather narrow, produced; anal sheath without armature, the inner, pointed ends being connected by a chitinous membrane with the tegumen to form a hood-like structure; claspers broad, chunky, sacculus slightly sinuate, terminating in a knob-like tuft of hair; aedeagus broad, long, vesica armed with an apical, shorter and a basal, longer pair of strong cornuti, with a ridged chitinous plate between.

*Genitalia, Female.* Ostium plate with long pointed lateral lobes; ostium broadly V-shaped; chitinous portion of ductus bursae long, broad, extending well beyond ends of lateral lobes; basal half of membranous portion of ductus expanded into an oblong sack beyond which is the inception of the ductus seminalis; signum large, stellate.

*Distribution.* Quebec, Meach Lake; Ontario, Ottawa, Biscotasing; Alberta, Edmonton; British Columbia, Agassiz.

*Food Plant.* Birch.

### ***Peronea nivisellana*** Wlshm.

Page 327, fig. 6; Page 332, fig. 1.

*Teras nivisellana* Walsingham, Ill. Lep. Het. Brit. Mus. IV, 2, Pl. LXI, fig. 3. 1879.
*Peronea nivisellana* Barnes and Busck, Cont. N. Hist. Lep. N. Am. IV (3), Pl. XXXII, fig. 9. 1920; Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta., 484. 1924.

A striking species with the dark head contrasting with the white thorax, the mottled whitish basal half of forewing and the dark purple-black costal patch and terminal suffusion, relieved inwardly by paler brown shades; the scale-tufts below the apex of the costal triangle are unusually prominent. There is little variation in the series under examination.

*Genitalia, Male* (One slide examined). Tegumen broad apically; anal sheath armed with a sharp ventro-apical spine; socii large, oval, projecting beyond lateral edge of tegumen; claspers narrow, bent upward apically, lower margin of sacculus gently sinuate, terminating in a small, slightly raised hair-tuft; aedeagus thin, armed with a short apical spine, vesica with one long, apical cornutus and another very minute one.

*Genitalia, Female* (One slide examined). Ostium plate with long, thin, straight, lateral lobes; ostium rather narrow, V-shaped, situated slightly above cephalic margin of plate; ductus bursae entirely membranous, somewhat expanded in proximal third, beyond which is the inception of the ductus seminalis; bursa large, with a long, band-like, spined signum.

*Distribution.* Quebec, Meach Lake; Ontario, Ottawa, Rostrevor, Biscotasing; Manitoba, Aweme; Alberta, Edmonton; British Columbia, Departure Bay.

*Food Plant. Crataegus* (*teste* Forbes).

In the remainder of the species under consideration, which belong in what may be termed the *ferrugana* group, the male genitalia show the lateral portions of the tegumen united by a chitinous membrane to the inner apices of the anal sheath, forming a hood-like structure. This will be simply referred to in future as "tegumen, hood-like." The species are all quite similar in general genitalic characters and are separated on the finer details, such as shape of socii, spining of vesica, etc.

### *Peronea cervinana* Fern.

Page 328, fig. 1; Page 332, fig. 2.

*Teras cervinana* Fernald, Trans. Am. Ent. Soc. X, 65. 1882.
*Teras americana* Fernald, Trans. Am. Ent. Soc. X, 66. 1882.
*Peronea cervinana* Barnes and Busck, Cont. N. Hist. Lep. N. Am. IV (3), Pl. XXXII, fig. 7. 1920.
*Peronea cervinana* Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 483. 1924.

The typical form is of a rather deep orange-brown color on forewing, at times finely reticulate with brown; the costal triangle is dark brown enclosing a striking white rectangular spot based on costa; in the form *americana* the ground color of the forewings is paler, more fawn-colored and the included white spot in the dark costal triangle is replaced by one of the same general color as the main portion of the wing. Another very marked form, of which only a single specimen has been examined, has the thorax largely whitish; in the forewing the basal third is white and the remainder of the wing a deep purple-brown, forming a very striking contrast.

*Genitalia, Male* (Five slides examined). Tegumen hood-like; anal sheath armed with a strong ventro-apical hood-like projection, socii erect, narrowly oval; claspers broad, chunky, the valvula projecting only slightly beyond apex of costa; margin of sacculus almost straight with slight chitinous flap beyond half and narrow apical hair-tuft; aedeagus moderate, strongly bent and slightly tapering with stout subapical spine, vesica with *two* strong cornuti and a shorter apical one.

*Genitalia, Female* (Two slides examined). Ostium plate with rather short lateral lobes; ostium broad, semicircular; ductus bursae shortly chitinized at proximal end, following which is a small, bulbous, membranous portion, containing a chitinous plate and narrowing distally; this is followed by a large chitinized sack with narrow band of fine spicules along one side, beyond which the ductus is narrowly tubular, membranous, and with the inception of the ductus seminalis just distad of chitinous sack; signum large, stellate.

*Distribution.* Quebec, Meach Lake, Kazabazua; Ontario, Ottawa; Alberta, Edmonton.

*Food Plant.* Unknown.

### *Peronea subnivana* Wlk.

Page 328, fig. 2; Page 332, fig. 3.

*Penthina subnivana* Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 376. 1863.
*Teras deflectana* Robinson, Trans. Am. Ent. Soc. II, 283, Pl. VII, fig. 71. 1869.
*Teras peculiana* Zeller, Verhandl. zool. bot. Ges. Wien, XXV, 210, Pl. VIII, fig. 1, 1875.
*Teras subnivana* Walsingham, Ill. Lep. Het. Brit. Mus. IV, 1, Pl. LXI, fig. 2. 1879.
*Peronea subnivana* Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 483. 1924.

A pale whitish or fawn-colored species with the forewings at times distinctly reticulate in light brown; the dark brown costal triangle may be solid or broken by a pale central patch. The costa of forewing is strongly concave (more so in the female sex than in the male) beyond half, and this feature should at once separate it from the allied *cervinana* and its forms.

*Genitalia, Male* (One slide examined). Very similar to *cervinana;* socii somewhat broader; clasper longer and narrower with the apex of valvula scarcely projecting and broadly rounded; sacculus margin rather more sinuate and without the small chitinous flap at half; aedeagus *much longer and narrower,* with smaller apical spine; armature of vesica consists of *one* long, stout spine and two short, but fairly stout ones.

*Genitalia, Female.* Ostium plate with lateral lobes slightly longer, narrower and more pointed than in *cervinana;* ostium slightly broader; in the ductus bursae the neck of the chitinized sack is shorter and broader, the sack itself less strongly chitinized and the spiculate band contains fewer spicules.

*Distribution.* Quebec, Chelsea, Meach Lake; Ontario, Ottawa.

*Food Plant. Vernonia* (*teste* Forbes).

### *Peronea braunana* n. sp.

Page 328, fig. 3; Page 332, fig. 4.

*Peronea ferrugana* Barnes and Busck (*nec* D. & S.), Con . N. Hist. Lep. N. Am. IV (3), Pl. XXXII, fig. 2. 1920.

Palpi and head smoky brown with an admixture of ochreous; thorax largely red-brown; primaries rather deep red-brown or purple-brown with a slight shading of gray scaling along inner margin; a strong purple-black costal triangle, broken somewhat on costa by a small patch of the ground-color; a faint line of white scaling borders the basal edge of this patch and there are a few slightly raised white and black scales scattered along its inner margin as well as a broken oblique line of similar scales directed from its outer edge toward outer margin of wing above anal angle; a moderately large tuft of black scales in fold near base. Fringes concolorous. Secondaries pale smoky, deeper outwardly. Expanse 15 mm.

*Genitalia, Male* (Three slides examined). Very similar to those of *cervinana.* Tegumen hood-like; anal sheath produced strongly beyond apex of tegumen; armed with a strong ventro-apical projection; socii upright, *narrower and more pointed* than in *cervinana;* claspers somewhat narrower than in *cervinana* with apex of valvula projecting further beyond costa; sacculus feebly emarginate with somewhat broader chitinous flap and a shorter apical hair-tuft; aedeagus thinner and longer than in *cervinana* armed with a shorter subapical spine, vesica with a single apical and a pair of median cornuti, all of equal length, and slightly less stout than the larger ones of *cervinana.*

*Genitalia, Female* (Two slides examined). Ostium plate with long, thin, parallel, lateral lobes; ostium much as in *cervinana*, broad, semicircular; basal section of ductus bursae similar to that of *cervinana* with short chitinized portion, followed by a bulbous expansion, containing a chitinized plate; the following sack-like expansion is, however, *entirely membranous;* signum moderately large, stellate.

*Holotype* ♂, Biscotasing, Ontario, September 9, 1931 (K. Schedl); No. 3751 in the Canadian National Collection, Ottawa.

*Allotype* ♀, Meach Lake, Quebec, September 29, 1904 (C. H. Young).

*Paratypes* 3 ♂, same data as holotype, September 12; 1 ♂, same data as allotype, September 23; 1 ♂, Ottawa Golf Club, Quebec, September 14 (F. P. Ide).

Further specimens have been studied from Kazubazua, Quebec; Algonquin Park, Ontario; Salmon Arm, and Alta Lake, British Columbia, the latter a striking form with the basal half of forewing white, as in a very similar form mentioned under *cervinana*.

The species has been generally misidentified as *ferrugana* or *semiannula* but, as is shown later, is distinct from either of these species.

*Food Plant.* Probably *Betula*.

### *Peronea kearfottana* n. sp.

Page 328, fig. 4; Page 332, fig. 5.

A small species with pale orange-yellow forewings, resembling considerably *minuta* Rob. Head and thorax light yellow-brown; primaries almost unicolorous orange-yellow with faint traces of reticulation and a general shiny appearance; secondaries light smoky with paler fringes. Expanse 15 mm.

In the allotype female and some paratype specimens there is a trace of a smoky oblique shade from costa at half to end of cell and in one specimen (hibernated) the forewing is suffused and reticulate with smoky over a pale creamy ground-color.

*Genitalia, Male* (Three slides examined). Tegumen hood-like; anal sheath armed with a strong ventro-apical hood-like projection; socii long, narrow, upright; ventral margin of sacculus gently sinuate with small, oval, terminal hair-tuft; aedeagus rather short, stout, bent; vesica with one or two small apical cornuti and a centrally situated pair, one of which is longer and stouter than the other.

*Genitalia, Female* (One slide examined). Ostium plate with short, pointed lateral lobes; ostium rather broad, semicircular; ductus bursae shortly membranous proximally, followed by an expansion into a large irregularly shaped sack, the neck of which is lightly chitinized, then strongly narrowed before the bursa, which is small, circular and with a small stellate signum.

*Holotype* ♂, Bobcaygeon, Ontario, June 20, 1932 (reared from *Myrica*) (J. McDunnough); No. 3746 in the Canadian National Collection, Ottawa.

*Allotype* ♀, same data, June 22, 1931.

*Paratypes* 2♂, Kazubazua, Quebec, July 19, 20 (F. P. Ide); 1 ♂, Biscotasing, Ontario, May 15 (K. Schedl); 2 ♀, Bobcaygeon, Ontario, June 21, July 10 (J. McDunnough); 1 ♀, Ottawa, Ontario, July 3 (C. H. Young).

*Food Plant. Myrica.*

### *Peronea simpliciana* Wlshm.

Page 328, fig. 5; Page 332, fig. 6.

*Teras simpliciana* Walsingham, Ill. Lep. Het. Brit. Mus. IV, 2, Pl. LXI, fig. 4. 1879.

The identification of this species is based on the excellent agreement of a small series in the Canadian National Collection with the original description and colored figure.

The size is small and the color of forewings whitish with a creamy or olivaceous tinge; there is a strong, blackish, flatly triangular blotch on costa at half which may or may not contain a pale centre, a black dot in the fold at one-third and a faint curved line of leaden scaling from the outer edge of costal triangle to outer margin above anal angle.

*Genitalia, Male* (Two slides examined). Tegumen hood-like; anal sheath with strong, narrow, truncate projection ventrally; socii upright, rather narrow and blunt apically; claspers moderately wide, sacculus gently sinuate with small, narrowly oval, apical hair-tuft; aedeagus narrow, apex bent at right angles, enlarged, *funnel-shaped;* vesica armed with two minute cornuti, one situated subapically, the other centrally.

*Genitalia, Female* (One slide examined). Ostium plate broad, lateral lobes incurved and sharply pointed; ostium broad, situated on cephalic margin of plate, ductus bursae with an enlarged, *bowl-shaped,* chitinized portion proximally followed by a long, thin membranous tube with the inception of the ductus seminalis at two-thirds. Signum stellate.

*Distribution.* Manitoba, Aweme.

*Food Plant.* Unknown.

### *Peronea forbesana* n. sp.

Page 328, fig. 6; Page 332, fig. 7.

Rather similar to *simpliciana* Wlshm., but larger and the forewings more variegated in appearance, due to the greater prominence of leaden-colored scaling.

Palpi, head and thorax light creamy-white, the latter shaded partially with olive-gray. Primaries with costal margin shaped as in *cervinana* Fern., creamy-white, shaded and suffused with olive-gray and with a prominent sharply defined, purplish-black, broadly triangular costal patch with slight admixture of brown scales, extending from costa at two-fifths to costa at four-fifths; basal area lightly and irregularly shaded with pale olive-gray, the outer edge of this area containing two minute black spots on cubital vein and in the fold; a slight shading of olive-gray on inner margin at half and a broad irregularly oblique band of olive-gray or leaden-colored scaling

from apex of costal patch to anal angle; a small, oval, olive-gray subapical patch and slight reticulations of similar color along outer margin; fringes olive-gray, paler outwardly. Scale-tufting not prominent, when present consisting of a small pale tuft below apex of costal patch in cell and a similar one in the fold at one-third. Expanse 15 to 17 mm.

*Genitalia, Male* (Four slides examined). Tegumen hood-like; anal sheath with the ventro-apical projection very weak; socii upright, long, slightly more pointed apically than in *simpliciana;* claspers broad, chunky, sacculus very slightly sinuate with narrowly oval apical hair-tuft; aedeagus rather long, thin, vesica armed with a single small apical cornutus, a small triangular piece of chitin with short spine (rose-thorn) and two centrally situated cornuti, slightly larger than the apical one.

*Genitalia, Female* (Two slides examined). Ostium plate with well-developed lobes, narrow and slightly divergent; ostium small, semicircular; ductus bursae *membranous and moderately broad proximally* to a point slightly beyond apices of lateral lobes, then somewhat constricted to form the neck of a long sack-like enlargement, this neck being lightly chitinized, and the membranous sack containing a narrow band of fine spicules; terminal portion of ductus very narrow. Signum stellate.

*Holotype* ♂, Bobcaygeon, Ontario, July 1, 1932 (bred from *Cornus*) (J. McDunnough); No. 3749 in the Canadian National Collection, Ottawa.

*Allotype* ♀, Fredericton, New Brunswick, May 1, 1928 (R. P. Gorham).

*Paratypes* 1 ♂, same data as holotype, July 6; 1 ♂, Meach Lake, Que., September 25, 1902 (C. H. Young); 1 ♂, Ottawa, Ontario, September 22, 1905 (C. H. Young); 2 ♂, 1 ♀, Aweme, Manitoba, September 7, October 4, April 3 (N. Criddle); 8 ♂, Edmonton, Alta., April 23–27, October 1 (K. Bowman), some of these deposited in Bowman's Collection.

Several somewhat larger specimens from the Kaslo region of British Columbia in the Canadian National Collection also belong here, one of these having been erroneously identified by Kearfott as *boscana* Fabr. a species which apparently does not occur in North America.

The present species rather resembles Kennel's figure of *lacordairana* Dup., but the genitalia are quite distinct, if Filipjev's figure of these organs in the above species be correct.

*Food Plant. Cornus.*

### *Peronea fuscana* B. & B.

Page 328, fig. 7; Page 332, fig. 8.

*Peronea fuscana* Barnes and Busck, Cont. N. Hist. Lep. N. Am. IV (3), 216. 1920.

The determination of the species is based on information kindly supplied by Mr. A. Busck regarding genitalic characters. It is rather difficult to separate from allied forms such as *cornana* McD. and *heindelana* Fern., but in general the forewings are more contrastingly marked than in either of these species. The ground color is at times quite pale gray and the blackish areas

at base of wing and along costa stand out in consequence quite prominently; there is frequently a strong blackish basal streak or patch present which is characteristic of the species and apparently does not occur in any of the allies.

*Fuscana* is very closely allied to the European *ferrugana* genitalically, the males showing the same spining of the vesica in both species; in *fuscana*, however, the socii appear considerably broader and the apical hair-tuft of the sacculus is stronger; in the female there are also minor differences which point to specific distinctness.

*Genitalia, Male* (Six slides examined). Tegumen hood-like; anal sheath with strong ventro-apical projection; socii upright, rather broad and blunt apically; sacculus feebly sinuate with a small projecting chitinous flap at half and a strong, broad, apical hair-tuft; claspers rather broad, aedeagus long, thin, bent; vesica armed with two small, single cornuti, separated by a small, shortly spined piece of chitin (rose-thorn).

*Genitalia, Female* (One slide examined). Ostium plate with long, nearly straight, lateral lobes, tapering apically; ostium narrow, semicircular; ductus bursae moderately broad and membranous for a short distance proximally, then faintly chitinized to a point near the inception of the ductus seminalis, this area showing faint spiculation distally; narrowing to a fine tube in distal half; signum large, heavily spined.

*Distribution.* Manitoba, Aweme; Alberta, Edmonton. Specimens have also been examined from Hampton, New Hampshire.

*Food Plant.* Unknown.

### *Peronea ferrugana* D. & S.

*P. Tortrix ferrugana* Denis and Schiffermuller, Syst. Verz. 128. 1776.
*Acalla ferrugana* Kennel, Palaearct. Tort. 93, Pl. V, figs. 40-50. 1909.
*Peronea ferrugana* Pierce and Metcalfe, Gen. Brit. Tort. 21, Pl. VIII. 1922.
*Peronea ferrugana* Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S. 522, Pl. XXV, fig. 3, Pl. XXVIII, fig. 3. 1930.
*Acleris ferrugana* Sheldon, Entomol. LXIV, 60. 1931.

This European species has long been recorded from North America, mainly on the erroneous supposition that *semiannula* Rob. was conspecific with it, which it is not. However, two specimens have been examined in the Canadian National Collection, a male from the Ottawa district and a female from Salt River, North West Territories, the genitalia of which seem to agree with those of European specimens and with the figures of Pierce and Filipjev. These two specimens are very similar to each other in the color and maculation of the forewings, but represent by no means the typical form of *ferrugana*, the ground-color being light gray, slightly speckled with brown specks and with a prominent, more or less tripunctate, brown, costal triangle; they approach closest to Kennel's Fig. 43. More material will be necessary before the specific record can be definitely established, but there seems no real reason why *ferrugana* should not be found in Canada, the food plant, birch, being of such general distribution.

The genitalia, as already stated, are very close to those of *fuscana* B. & B. and are very adequately figured by Pierce; the chief differences in the male genitalia have been commented on under *fuscana;* in the female organs, the lateral lobes of the ostium plate are shorter, thinner and more widely separated; the ostium is narrower; the chitinized portion of the ductus bursae shows a peculiar, crenulate appearance proximally, and is without indication of spicules distally.

### *Peronea semiannula* Rob.

Page 328, fig. 8; Page 332, fig. 9.

*Teras semiannula* Robinson, Trans. Am. Ent. Soc. II, 282, Pl. VII, fig. 70. 1869.
*Peronea stadiana* Barnes and Busck, Cont. N. Hist. Lep. N. Am. IV (3) 217. 1920.

The species has been very generally confused and associated with the birch-feeder. A critical study of the original description and figure led to the belief that this association was incorrect and further confirmation was recently secured by Mr. W. J. Brown who, on a visit to the Academy of Natural Sciences, Philadelphia, compared specimens with what appears to be the original type and made careful notes on same. It would seem that the name should be applied to the maple-feeder, *stadiana* B. & B. unfortunately falling as a synonym; of *stadiana* there are not only Paratypes, but also a long series of topotypical specimens in the Canadian National Collection.

The usual color of the forewing in *semiannula* is a light red-brown, but this may pale to fawn color and even occasionally to dull smoky gray; the costal triangle is frequently composed of red-brown scales, is rather indistinct and generally tripunctate in character; it may, however, be dark brown, distinct and either enclosing a pale costal spot or broken up into three, dark, separated spots. Occasional specimens occur with a distinct strigate appearance, due to the presence of small, black streaks in the basal and terminal areas of wing.

*Genitalia, Male* (Six slides examined). Tegumen hood-like; anal sheath with moderately strong ventro-apical projection; socii upright, somewhat shorter and narrower than in *fuscana* and much as in *ferrugana;* claspers rather short and broad; ventral margin of sacculus slightly angled at base, then feebly sinuate; no chitinous flap, and smaller apical tuft than in *fuscana;* aedeagus narrow, bent, vesica armed with a pair of short apical cornuti and a second longer pair, situated more centrally and with a small chitinous plate between them.

*Genitalia, Female* (Four slides examined). Lateral lobes of ostium plate moderately long, wide apart, slightly divergent; ostium small, semicircular; ductus bursae almost entirely membranous, with the exception of a short, lightly chitinized ring near proximal end, beyond which is a long sack-like enlargement; terminal portion narrow with inception of ductus seminalis close to bursa; signum large, stellate.

*Distribution.* Quebec, Meach Lake; Ontario, Ottawa; Alberta, Edmonton; British Columbia, Salmon Arm. Also occurs at Hazelton, Pennsylvania.

*Food Plant.* Maple (probably).

## *Peronea gallicolana* Clem.

Page 328, fig. 9; Page 332, fig. 10.

*Peronea gallicolana* Clemens, Proc. Ent. Soc. Phil. III, 516. 1864; Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 487. 1924.
*Acleris heindelana* Fernald, Am. Nat. XXXIX, 870. 1905.

The typical form of this species, to judge by the original description, is one in which the forewings are rather pale grayish with a distinct and sharply defined, black-brown, costal triangle; the writer has examined a single specimen from Trenton, Ont., which matches this description. The usual form in Canada is the one described later as *heindelana;* the forewings are largely suffused with smoky brown, at times with a slight ruddy tinge; the dark costal triangle is in consequence not prominent, generally better defined in the females than in the males.

*Genitalia, Male* (Five slides examined). Tegumen hood-like; anal sheath projecting strongly beyond apex of tegumen and with a strong ventro-apical projection; socii upright, rather broader and longer than in allied species; claspers distinctly longer and narrower than in *fuscana;* sacculus feebly sinuate with small chitinous flap at half and weak apical hair-tuft; aedeagus thin, vesica armed with a group of two or three small apical cornuti and a small piece of chitin as well as two further cornuti, slightly larger than the apical ones and more centrally located.

*Genitalia, Female* (Four slides examined). Ostium plate rather broad with short, parallel, lateral lobes; ostium small, semicircular, situated higher on the plate than in allied species; ductus bursae almost entirely membranous, narrow proximally for a short distance, then expanding into a long oval sack, which shows a slight chitinous thickening and a faint crenulation at one side proximally; beyond the sack is the inception of the ductus seminalis, the remainder of the ductus bursae being short and narrow, signum moderately large, stellate.

*Distribution.* Ontario, Ottawa, Trenton, Ingersoll; Manitoba, Aweme.

*Food Plant.* Cone-gall on willow.

## *Peronea cornana* McD.

Page 328, fig. 10; Page 332, fig. 11.

*Peronea cornana* McDunnough, Can. Ent. 207. 1933; *id.* Can. J. Research, IX, 509. 1933.

The general even olive-gray color of the primaries is rather characteristic of the species. The series on which the name was based evidently belonged to the first generation; specimens taken late in the fall are somewhat larger. In the male genitalia the decumbent socii at once separate the species from its allies.

*Genitalia, Male* (Six slides examined). Tegumen rather broad, hood-like; anal sheath strongly projecting beyond apex of tegumen, ventro-apical projection much reduced; socii *decumbent,* broadly oval; claspers rather long and narrow, sacculus feebly sinuate, apical hair-tuft well developed; aedeagus

moderately thin, vesica armed with two apical cornuti, arranged one behind the other, and a pair (sometimes three) of similar-sized cornuti, situated more centrally.

*Genitalia, Female* (Four slides examined). Ostium plate rather narrow, lateral lobes very short, at times scarcely projecting beyond margin of plate; ostium small, semicircular; ductus bursae membranous and moderately broad proximally for a short distance, then feebly chitinized, this chitinous portion forming the neck of a long membranous sack-like expansion, narrowing to a thin tube shortly before entrance into bursa; signum moderately large, stellate.

*Distribution.* Quebec, Aylmer; Ontario, Bobcaygeon; Manitoba, Aweme; Alberta, Edmonton.

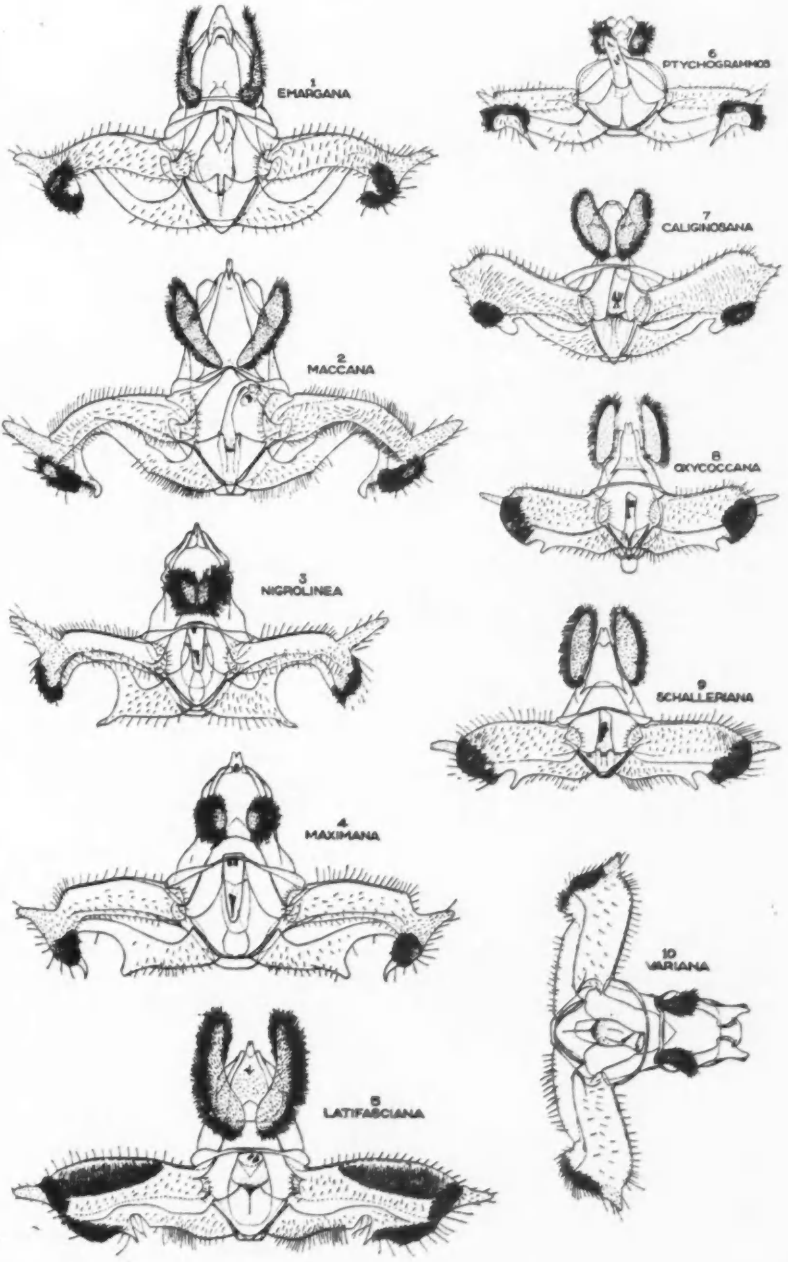
*Food Plant. Cornus.*

### *Peronea contaminana* Hbn.

*Tortrix contaminana* Hubner, Eur. Schmett. Tort. fig. 142. 1797.
*Acalla contaminana* Kennel, Palaearc. Tort. 99, Pl. V, figs. 59-61. 1908.
*Peronea reticulata* Pierce and Metcalfe, Gen. Brit. Tort. 23, Pl. IX. 1922.
*Aleimma contaminana* Sheldon, Entomol. LXIV, 79. 1931.

In conclusion it might be well to record the occurrence of this European species at Yarmouth, Nova Scotia, where it was found destructive to hawthorn hedges in 1924; it is probably a quite recent introduction.

The subfalcate primaries separate the species at once from other members of the genus and there may be, on this account, some justification for Sheldon's placement in the genus *Aleimma*. The general color of primaries is light to dark brown, more or less reticulate and spotted with darker shades.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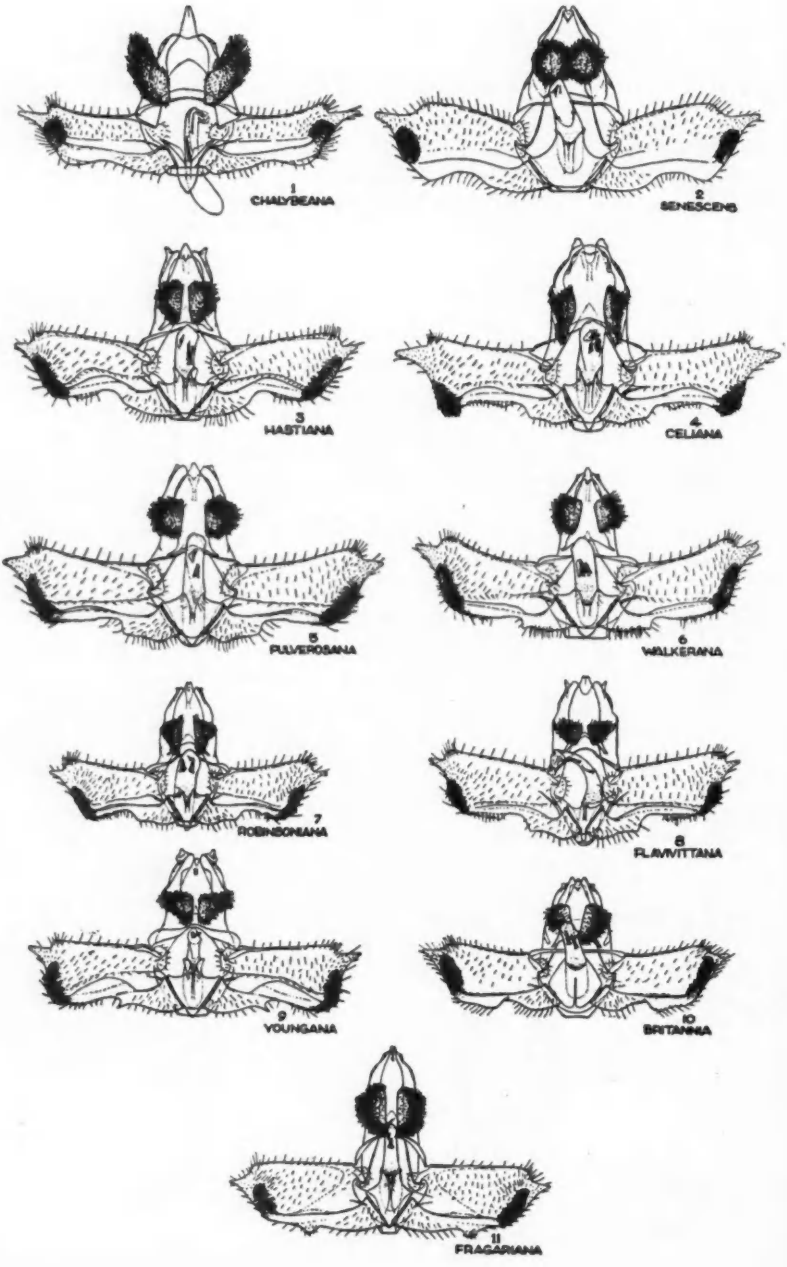
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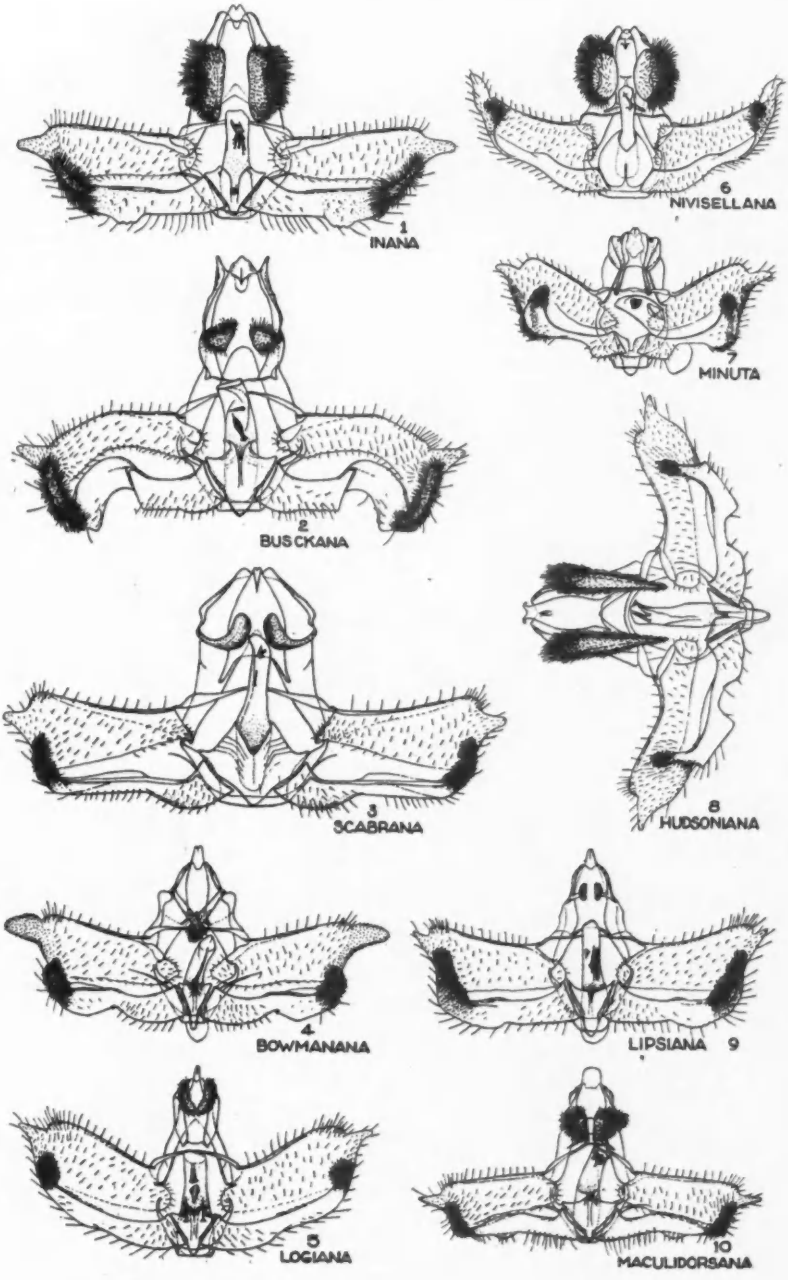
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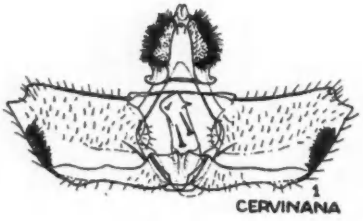
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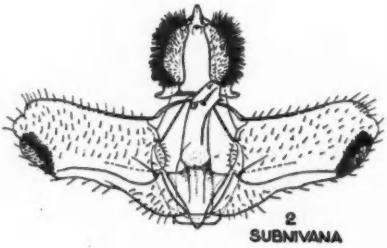
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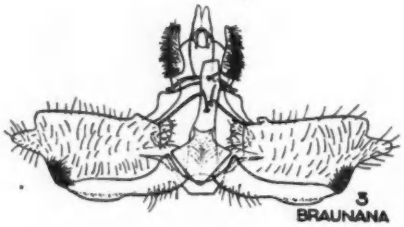
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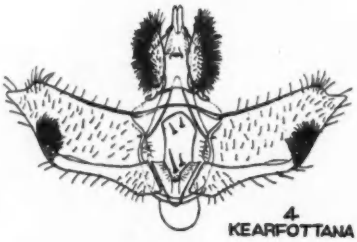
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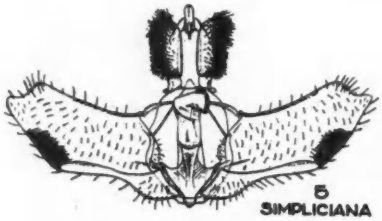
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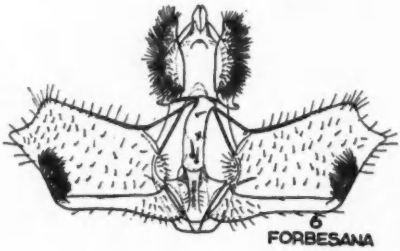
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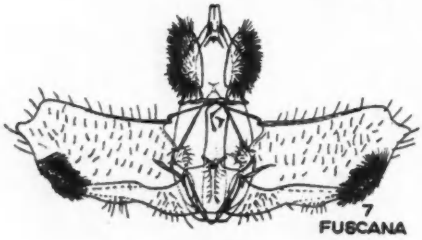
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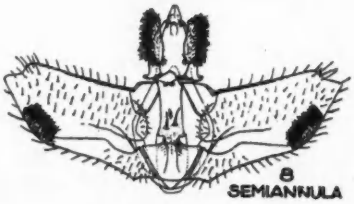
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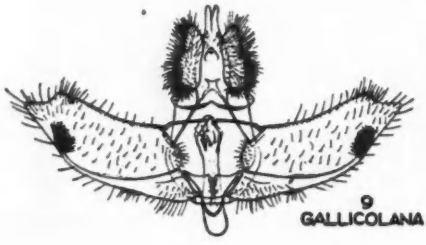
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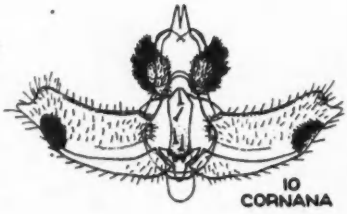
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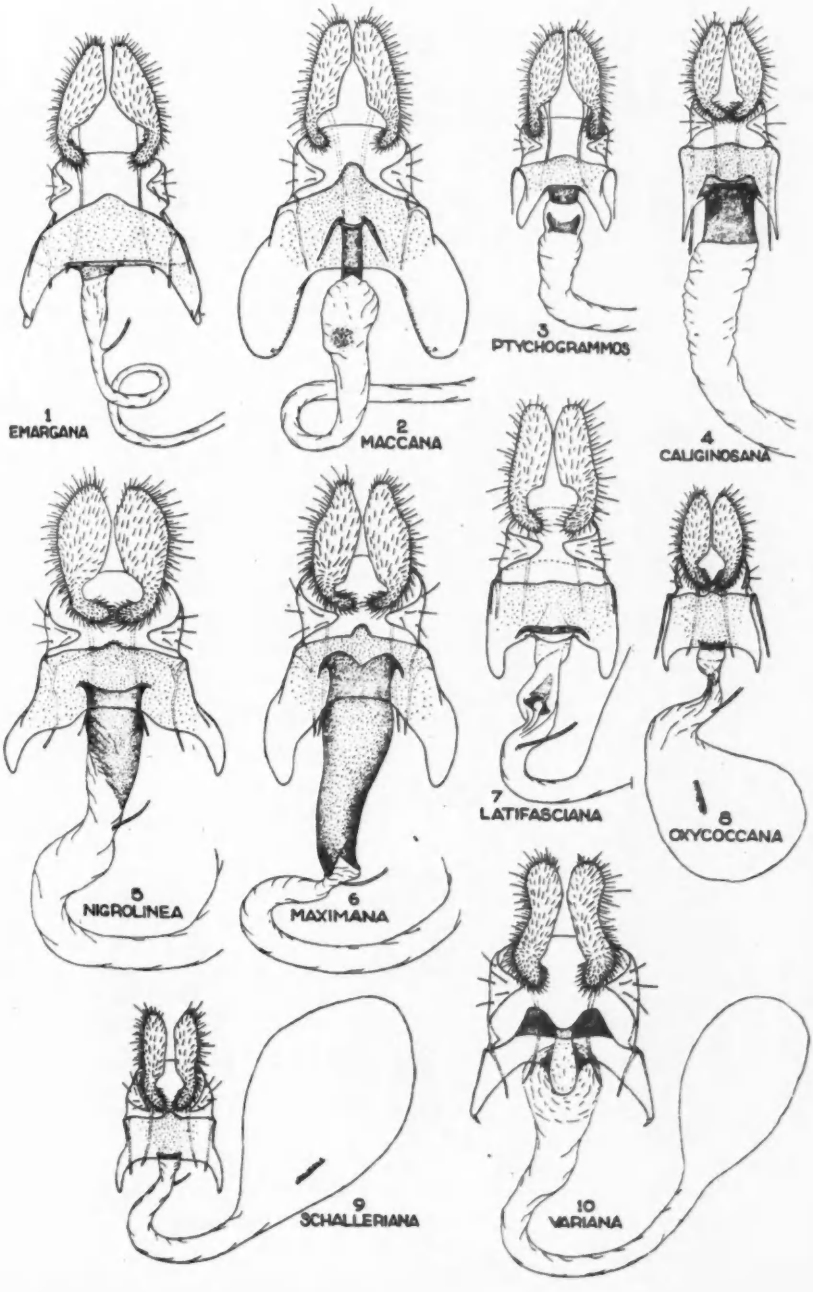
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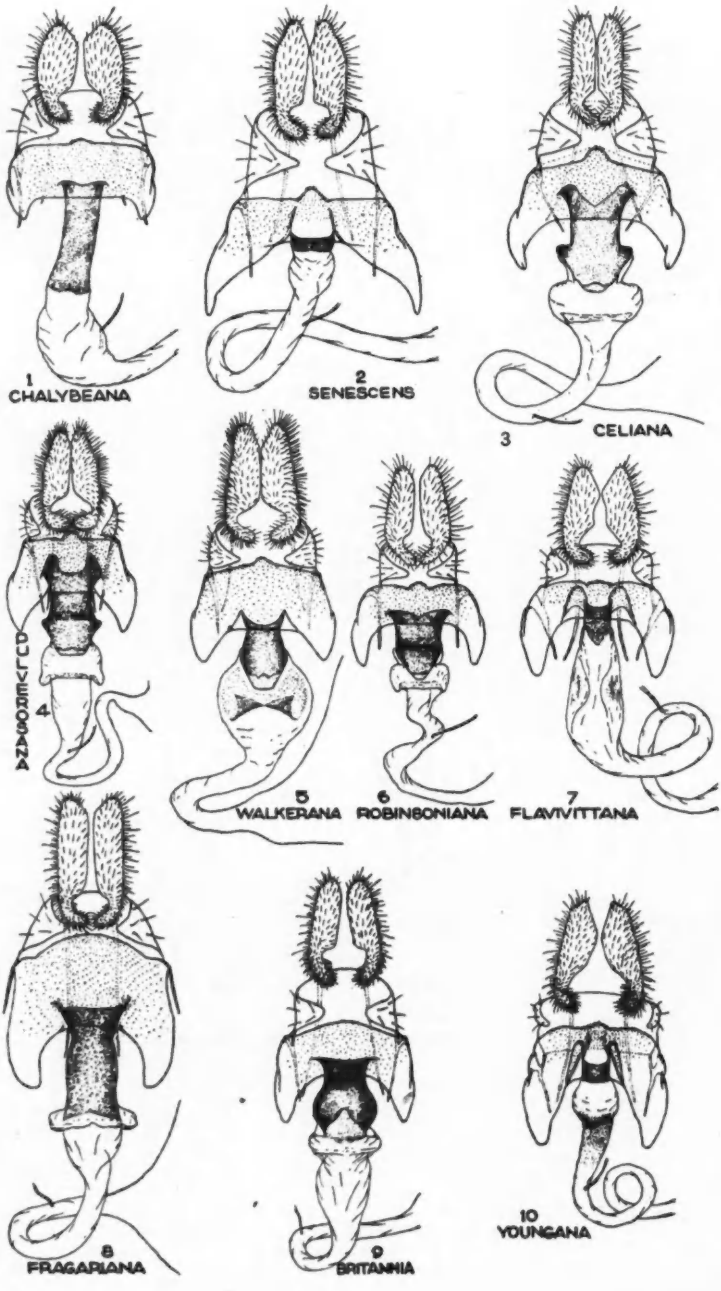
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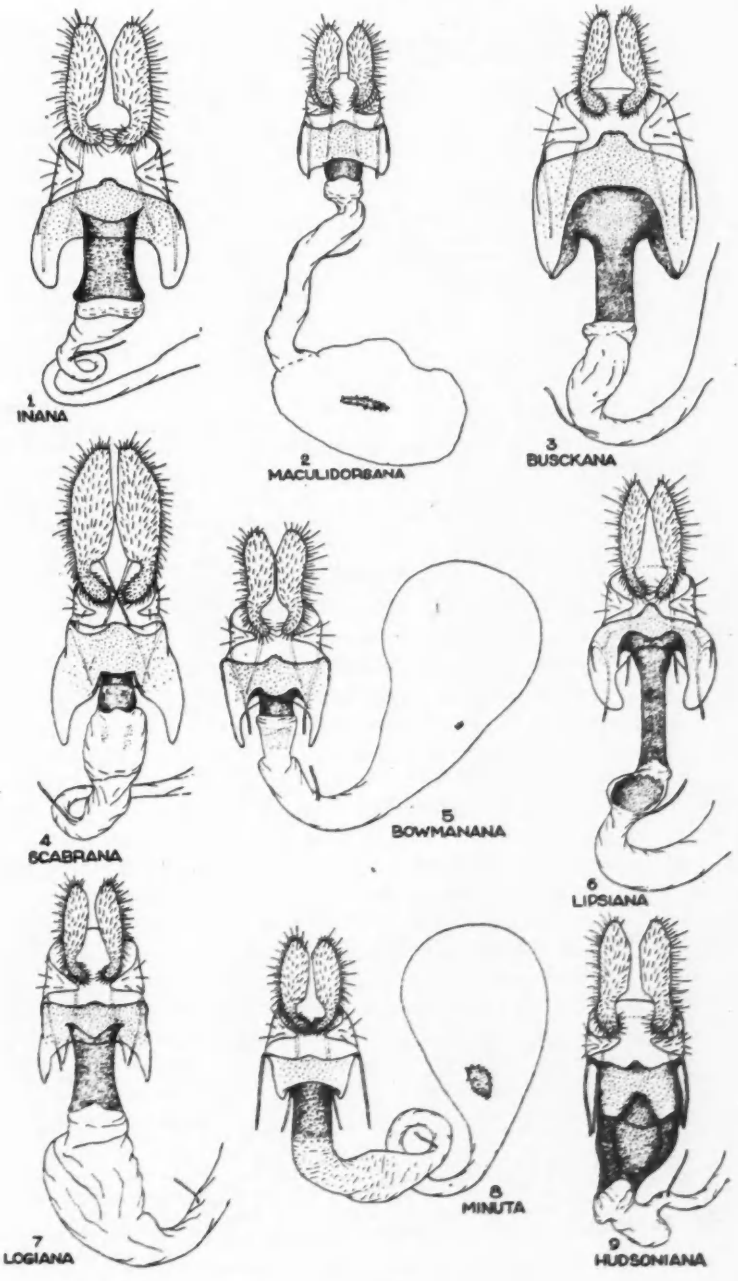
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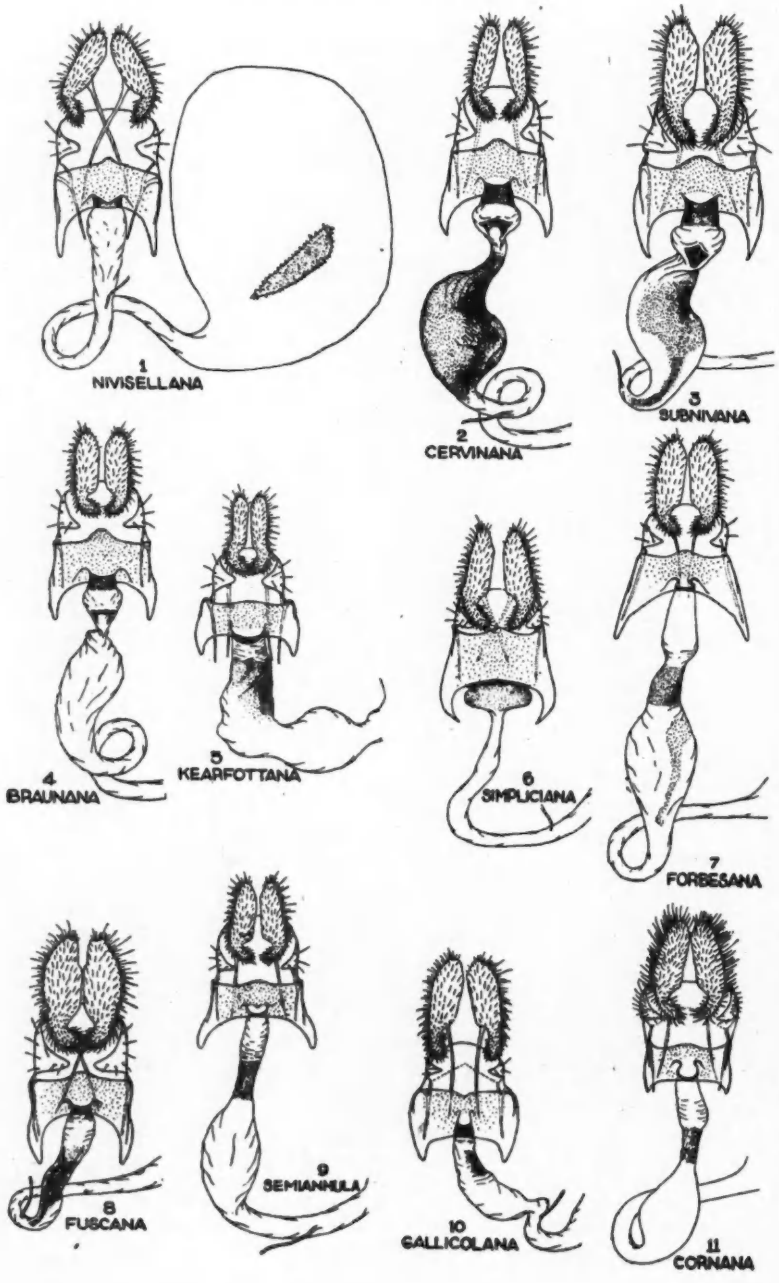
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FORBESANA
8
FUSCANA
9
SEMIANNULA
10
GALLICOLANA
11
CORNANA

# $F_2$ ENDOSPERM DEVELOPMENT IN RELATION TO BREEDING TECHNIQUE WITH INTER-SPECIFIC WHEAT CROSSES[1]

BY J. B. HARRINGTON[2] AND J. B. MARSHALL[3]

## Abstract

A comparative study was made of three interspecific wheat crosses with regard to the relationship between seed plumpness and plant character in the $F_2$ generation. In general, the degree of *vulgare*-ness of the plants was associated with the degree of shrunkenness of the seeds, the most shrunken seeds producing the most *vulgare*-like plants. However, many shrunken seeds did not produce *vulgare*-like plants and many plump seeds did not produce plants resembling the 14-chromosome parental variety. Desirable *vulgare*-like plants were obtainable from each plumpness class, the largest proportion being from the shrunken seeds. Plants from shrunken seeds that were germinated under ideal conditions proved to be no more *vulgare*-like than those which survived sowing under rigorous conditions in the field. The *durum* × *vulgare* cross was high in percentage of shrunken seeds and relatively high in the proportion of *vulgare*-like plants from plump seeds. The *dicoccum* × *vulgare* cross was low in proportion of shrunken seeds and had relatively few *vulgare*-like plants from plump seeds. In these crosses it would appear unnecessary for the breeder to pay attention to differences in $F_2$ seed plumpness. The *persicum* × *vulgare* cross showed a fair proportion of shrunken seeds but had relatively few *vulgare*-like plants from plump seeds. In this cross it would appear advisable to have an abundance of $F_2$ seeds and sow only the more shrunken half of them.

## Introduction

Hybridization as a plant breeding method is necessarily somewhat complicated and practical methods which tend to simplify and reduce the work in connection with handling large hybrid populations deserve every consideration. Wide species crosses are now used extensively for obtaining various character combinations which are not obtainable from narrow crosses. The necessity for having very large hybrid populations where wide species crosses are concerned has been shown by various investigations.

The breeder working with a large population of a wide cross in wheat has available several methods of procedure and it is not always easy to decide which course to pursue. After parental varieties have been selected, he is faced with the choice of which to use as the female parent. He also has to decide how many $F_1$ seeds he needs, how to sow the $F_1$, how to handle the $F_2$ seeds, and how to carry on from the growing $F_2$ to his final objective. Experience has shown that widely different results may be obtained with different breeding procedures. As the cost factor is important it is necessary to use an efficient technique, otherwise the size of population required to achieve a satisfactory outcome might not be possible with the available funds.

It is obvious that effective breeding work rests upon an adequate foundation of facts concerning technique. There is much discussion as to the relative

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*Contribution from the laboratories of the University of Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms a part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Manitoba, Saskatchewan and Alberta.*

[2] *Professor of Field Husbandry, University of Saskatchewan.*
[3] *Graduate Assistant, University of Saskatchewan.*

merits of different breeding procedures. The questions that arise very often concern the relative values of the shrunken and plump $F_2$ seeds. The present study was made with the object of contributing to the technique of handling wide wheat crosses by furnishing answers to some of these questions.

This study is a continuation and an enlargement of a previous study by Harrington (3) in which the relationship between endosperm development and morphologic characters in the $F_2$ generation of the cross Vernal emmer × Marquis was investigated. The previous study showed the essential differences between plants and populations obtained from shrunken $F_2$ seeds and those grown from plump $F_2$ seeds. The results were striking and the question arose: may similar results be expected in other wide crosses? To answer this question a study was made of two quite different crosses, one being *durum* × *vulgare* and the other *persicum* × *vulgare*.

## Materials

The material used was obtained from crosses made in connection with actual breeding projects. These crosses involved four species in the following crosses: Iumillo × Marquis, Black Persian × Marquis and Vernal × Marquis. The *durum*, Iumillo Sask. III-25-56, is a strain of high purity selected at the University of Saskatchewan and tracing back to stock originally received from the United States Department of Agriculture. The Marquis crossed with Iumillo is Sask. No. 7, a strain descended directly from the original introduction of Marquis from the Dominion Experimental Farm System of Canada and originated at the University of Saskatchewan by mass selection. The *persicum*, Black Persian Sask. No. 1801, is a purified strain selected at the University of Saskatchewan from the original stock obtained from the United States Department of Agriculture. The Marquis crossed with Black Persian is Sask. No. 1221, a uniform strain from a pedigree selection made at the University of Saskatchewan. The *dicoccum* × *vulgare* cross was described by Harrington (2).

## Procedure

A procedure similar to that used by Harrington (2) in 1929 and 1930 on the cross Vernal (*T. dicoccum*) × Marquis (*T. vulgare*) was followed in the present study. Results from the previous study are therefore comparable to those of the present study and will be used here to represent the combination *dicoccum* × *vulgare*.

A random sample of 702 $F_2$ seeds from the *durum* × *vulgare* cross (Iumillo × Marquis) was divided into three plumpness classes as follows: Plump (Class *A*), slightly shrunken (Class *B*) and shrunken (Class *C*). The sample consisted of 30.9% of Class *A*, 34.3% of Class *B* and 34.8% of Class *C*. The material was hand sown in the breeding nursery in 5-ft. rows, 1 ft. apart, with Marquis and Iumillo every sixth and seventh row. Stands were taken three times, commencing June 8, at intervals of seven days. At maturity each group and parental checks were harvested separately by pulling the

individual plants. A total of 264 $F_2$ plants was studied in the laboratory with respect to 13 morphologic characters in which the parent varieties differed markedly. A description of the two varieties for each character is given in Table I.

TABLE I

DESCRIPTION OF THE PARENT VARIETIES FOR THE CHARACTERS STUDIED

| Character | Marquis (*vulgare*) | Vernal (*dicoccum*) | Iumillo (*durum*) | Black Persian (*persicum*) |
|---|---|---|---|---|
| Spike form (width ratio of lateral side to dorsi-ventral side) | 0.75–0.83 | 1.5–1.7 | 0.77–0.85 | |
| Spike compactness (average length of 10 central internodes) | 5.1–5.4 mm. | 3.4–3.8 mm. | 3.5–4.0 mm. | |
| Stem hollowness (taken 2 cm. below the collar) | Hollow with thin walls | Solid | Solid | Solid |
| Stem thickness (taken 2 cm. below collar) | 1.9–2.1 mm. | 0.9–1.0 mm. | 1.3–1.6 mm. | 1.4–1.7 mm. |
| Lower width of rachis segment | 1.9–2.1 mm. | 0.9–1.0 mm. | 1.5–1.7 mm. | 1.0–1.2 mm. |
| Rachis hairiness (marginal) | Hairs present, full length | No hairs | Hairs bristle-like at upper end of inte node | Hairs bristle-like at upper end of internode |
| Rachis articulation | Difficult | Easy | Difficult | Difficult |
| Shoulder width | Wide | Narrow | Narrow | Narrow |
| Glume length-width ratio | Length almost twice width | Length three times width | Length three tin es width | Length three times width |
| Keel sharpness (angle shown by cross section of lower half) | Obtuse | Acute | Acute to 90° | |
| Beak character | Short, blunt | | Prominent and curved | |
| Keel prominence | Slight prominence fro.n beak to base of glume | | Very prominent | |
| Brush character | Numerous hairs | | Few short hairs | |
| Seed character | Short, plump, with round cheeks and wide deep crease | Long, narruw with angular cheeks and narrow shallow crease | Medium long, with angular cheeks; narrow shallow crease and pointed germ | |
| Glume adherence | Easily detached | Detached with difficulty | | |
| Awning | Tip awned | Fully awned | | |

A similar procedure was followed in the study of the *persicum* × *vulgare* cross. A random lot of 402 $F_2$ seeds was divided according to the three plumpness classes. There was 17.6% of Class *A* seed, 35.6% of Class *B* and 46.8% of Class *C*. Stands were taken June 8, 16 and 24. *Persicum* and *vulgare* differ by fewer distinctive morphologic characters than do *durum* and *vulgare*. Seven characters were used in this study. The parent varieties are described with respect to these characters in Table I. Other characters could have been used, but the differences were not sufficiently uniform and definite to afford a satisfactory analysis.

The hybrid plants of each cross were classified for each of the contrasted characters listed in Table I into five groups, *viz: D*, those resembling the 14-chromosome parent; *V*, those resembling the *vulgare* parent; *I*, those intermediate between *D* and *V*; *ID*, those intermediate between *I* and *D*; and *IV*, those intermediate between *I* and *V*. Most of the data were obtained through visual comparisons of the hybrid with plants of the parental varieties grown close by. Uniform class intervals were maintained for each character as far as it was possible to do so. Occasionally checks on the accuracy of the work were made by actual measurements. In the case of a few characters one or both of the end classes exceeded the interval of the other classes. This was due to the appearance of some forms beyond the parental range and the placing of these segregates in the parental classes rather than in additional classes.

The data on plant character were tabulated for each cross according to the plumpness classes of the seed. For convenience the plants arising from seed plumpness Class *A* will be referred to as Class *A*, those from Class *B* seed as Class *B* and those from Class *C* as Class *C*.

## Results on the Relationship between Seed Plumpness and Morphologic Plant Characters in the $F_2$ of the Crosses *durum* × *vulgare*, *persicum* × *vulgare* and *dicoccum* × *vulgare*

The results on the three crosses should be presented together as they all bear upon the same fundamental problem. Therefore, although the *dicoccum* × *vulgare* data have been published, they will be considered here in relation to the results from the other crosses.

### *Proportion of each Plumpness Class in the Random Samples*

The separation of each $F_2$ random sample of seeds into three plumpness classes gave the results shown in Table II. The differences between the crosses are quite large. The proportion of shrunken seeds in the *dicoccum* × *vulgare* (this will be referred to as *VM*) $F_2$ is very much lower and the proportions of plump and slightly shrunken seeds are much higher than in

TABLE II

PROPORTION OF EACH PLUMPNESS CLASS IN THE RANDOM SAMPLES USED

| Cross | Cross designation | Distribution of seeds according to plumpness | | | | | |
|---|---|---|---|---|---|---|---|
| | | Class *A* | | Class *B* | | Class *C* | |
| | | No. | % | No. | % | No. | % |
| *durum* × *vulgare* | *IM* | 217 | 30.9 | 241 | 34.3 | 244 | 34.8 |
| *persicum* × *vulgare* | *MP* | 71 | 17.6 | 143 | 35.6 | 188 | 46.8 |
| *dicoccum* × *vulgare* | *VM* | 2902 | 55.5 | 2144 | 41.0 | 183 | 3.5 |
| Average | | | 34.7 | | 37.0 | | 28.3 |

the other crosses. The *VM* $F_2$ was grown in the wet season of 1928, whereas the other $F_2$ populations were grown in the distinctly dry season of 1930. Plants grown in 1928 had an excellent opportunity to produce kernels with the maximum amount of endosperm development allowed by their chromosome condition. In 1930 the reverse was true. A part of the differences under consideration may be due to this dissimilarity in growing conditions. The difference between the *durum* × *vulgare* and *persicum* × *vulgare* distributions may be due in part to the difficulty of classifying the seeds of widely unlike crosses in the same way.

*Seedling emergence*

The seedling emergence for the three crosses is shown in Table III. Emergence from Class *A* seeds approached that of the parent varieties in the *VM* $F_2$ and in the *persicum* × *vulgare* $F_2$ (this will be called the *MP* cross) but was low, 52.5%, in the *durum* × *vulgare* $F_2$ (this will be referred to as the *IM* cross). Emergence for Class *B* averaged somewhat lower than for Class *A*. Class *C* emergence was very low in all cases, being approximately half that of Class *A*. The Class *A* emergence in the *IM* cross is strikingly lower than in the *MP* cross which was grown during the same season.

TABLE III

PERCENTAGE SEEDLING EMERGENCE OF THE THREE INTERSPECIFIC CROSSES

| Year of study | Cross | Parental variety $F_2$ population | | | | |
|---|---|---|---|---|---|---|
| | | *Vulgare* | 14-chrom. species | Class *A* | Class *B* | Class *C* |
| 1931 | *durum* × *vulgare* (*IM*) | 91.6 | 88.4 | 52.5 | 53.9 | 25.4 |
| 1931 | *persicum* × *vulgare* (*MP*) | 98.0 | 80.0 | 80.3 | 62.2 | 28.7 |
| 1929-30 | *dicoccum* × *vulgare* (*VM*) | 71.5 | 67.5 | 64.0 | 58.0 | 35.5 |
| | Average | 87.0 | 78.6 | 65.6 | 58.4 | 29.9 |

*Comparison of Classes A, B and C for Plant Characters*

The distributions of the $F_2$ plants of the *IM* cross with respect to 13 morphologic characters are given in Table IV for each of the seed plumpness classes. The distributions for the three plumpness classes are dissimilar. Considering the average distribution for all characters, Class *A* has nearly twice as large numbers in the *D* and *ID* categories as in *V* and *IV*. Class *C* has about equal numbers in *D* and *ID* as in *V* and *IV*. Class *B* is intermediate with a closer approach to Class *A* than to Class *C*.

The results on the *MP* cross are given in Table V. Here the same tendency is shown except that it is more pronounced, especially in the Class *A* and Class *B* distributions.

TABLE IV

DISTRIBUTION OF THE $F_2$ PLANTS OF THE *durum* × *vulgare* CROSS WITH RESPECT TO EACH OF 13 MORPHOLOGIC CHARACTERS

| Seed plumpness class | Character category | Spike form | Spike compactness | Stem hollowness | Stem thickness | Rachis width | Rachis hairiness | Shoulder width | Glume l-w ratio | Keel sharpness | Beak character | Keel prominence | Brush character | Seed character | Average for all characters |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A* | *D* | 39 | 64 | 41 | 38 | 24 | 54 | 50 | 48 | 42 | 25 | 29 | 12 | 15 | 37 |
| | *ID* | 16 | 4 | 19 | 14 | 18 | 8 | 16 | 13 | 16 | 18 | 19 | 17 | 42 | 17 |
| | *I* | 31 | 8 | 21 | 17 | 20 | 9 | 5 | 12 | 13 | 20 | 28 | 23 | 7 | 16 |
| | *IV* | 8 | 3 | 13 | 11 | 15 | 5 | 10 | 11 | 15 | 13 | 16 | 10 | 19 | 11 |
| | *V* | 8 | 23 | 8 | 22 | 25 | 26 | 21 | 18 | 16 | 26 | 10 | 28 | 7 | 18 |
| *B* | *D* | 31 | 56 | 34 | 46 | 26 | 58 | 41 | 41 | 31 | 30 | 28 | 15 | 19 | 35 |
| | *ID* | 12 | 7 | 27 | 14 | 20 | 3 | 25 | 14 | 17 | 12 | 25 | 25 | 31 | 18 |
| | *I* | 27 | 11 | 34 | 16 | 24 | 5 | 8 | 14 | 21 | 19 | 27 | 10 | 16 | 18 |
| | *IV* | 20 | 12 | 6 | 11 | 16 | 7 | 16 | 15 | 23 | 16 | 22 | 15 | 26 | 16 |
| | *V* | 17 | 21 | 6 | 20 | 21 | 34 | 17 | 23 | 15 | 30 | 5 | 30 | 3 | 19 |
| *C* | *D* | 10 | 18 | 11 | 15 | 5 | 21 | 10 | 15 | 15 | 17 | 10 | 4 | 5 | 12 |
| | *ID* | 6 | 6 | 11 | 7 | 13 | 2 | 18 | 12 | 9 | 2 | 13 | 10 | 14 | 9 |
| | *I* | 9 | 6 | 20 | 4 | 14 | 3 | 9 | 4 | 11 | 12 | 14 | 9 | 8 | 9 |
| | *IV* | 15 | 8 | 8 | 13 | 11 | 9 | 13 | 12 | 11 | 9 | 10 | 10 | 12 | 11 |
| | *V* | 15 | 17 | 5 | 16 | 12 | 20 | 5 | 12 | 9 | 15 | 8 | 13 | 6 | 12 |

TABLE V

DISTRIBUTION OF THE $F_2$ PLANTS OF THE *persicum* × *vulgare* CROSS WITH RESPECT TO SEVEN MORPHOLOGIC CHARACTERS

| Seed plumpness class | Character category | Stem thickness | Stem hollowness | Rachis width | Rachis hairiness | Shoulder width | Glume l-w ratio | Beak character | Average for all characters |
|---|---|---|---|---|---|---|---|---|---|
| *A* | *D* | 26 | 25 | 16 | 34 | 35 | 26 | 1 | 23 |
| | *ID* | 4 | 13 | 19 | 3 | 9 | 7 | 5 | 9 |
| | *I* | 11 | 8 | 7 | 3 | 3 | 7 | 7 | 7 |
| | *IV* | 5 | 2 | 5 | 3 | 2 | 5 | 14 | 5 |
| | *V* | 4 | 2 | 3 | 7 | 1 | 5 | 23 | 6 |
| *B* | *D* | 40 | 25 | 17 | 39 | 37 | 31 | 8 | 27 |
| | *ID* | 6 | 23 | 18 | 6 | 19 | 10 | 5 | 12 |
| | *I* | 8 | 10 | 16 | 5 | 4 | 6 | 9 | 8 |
| | *IV* | 5 | 7 | 10 | 3 | 5 | 9 | 11 | 7 |
| | *V* | 10 | 4 | 8 | 16 | 4 | 13 | 36 | 13 |
| *C* | *D* | 25 | 8 | 2 | 13 | 26 | 20 | 0 | 13 |
| | *ID* | 7 | 18 | 11 | 10 | 16 | 10 | 3 | 11 |
| | *I* | 7 | 12 | 24 | 13 | 5 | 1 | 4 | 9 |
| | *IV* | 7 | 8 | 9 | 4 | 3 | 10 | 5 | 7 |
| | *V* | 5 | 5 | 5 | 11 | 1 | 10 | 39 | 11 |

Table VI shows the distributions of the *VM* cross. The preponderance of *D* and *ID* over *V* and *IV* in all three plumpness classes is even stronger here than in the *IM* cross.

TABLE VI

DISTRIBUTION OF THE $F_2$ PLANTS OF THE *dicoccum* × *vulgare* CROSS WITH RESPECT TO 12 MORPHOLOGIC CHARACTERS

| Seed plumpness class | Character category | Spike form | Spike compactness | Stem hollowness | Rachis articulation | Rachis width | Stem thickness | Glume adherence | Rachis hairiness | Shoulder width | Glume shape | Keel sharpness | Awning | Average for all characters |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A* | *D* | 17 | 40 | 21 | 14 | 18 | 11 | 6 | 30 | 27 | 23 | 16 | 15 | 20 |
| | *ID* | 16 | 0 | 12 | 23 | 12 | 15 | 8 | 11 | 13 | 12 | 8 | 7 | 11 |
| | *I* | 8 | 1 | 10 | 6 | 9 | 14 | 12 | 3 | 5 | 9 | 9 | 2 | 7 |
| | *IV* | 4 | 1 | 3 | 1 | 5 | 5 | 15 | 1 | 1 | 2 | 5 | 6 | 4 |
| | *V* | 1 | 4 | 0 | 2 | 2 | 1 | 5 | 1 | 0 | 0 | 8 | 16 | 3 |
| *B* | *D* | 37 | 58 | 14 | 28 | 18 | 26 | 22 | 40 | 51 | 37 | 32 | 25 | 32 |
| | *ID* | 29 | 8 | 27 | 19 | 21 | 20 | 18 | 22 | 22 | 34 | 21 | 8 | 21 |
| | *I* | 15 | 10 | 26 | 23 | 25 | 23 | 25 | 11 | 13 | 15 | 18 | 17 | 18 |
| | *IV* | 8 | 5 | 16 | 15 | 19 | 14 | 15 | 8 | 3 | 3 | 10 | 28 | 12 |
| | *V* | 1 | 9 | 7 | 5 | 7 | 7 | 10 | 9 | 1 | 1 | 9 | 12 | 7 |
| *C* | *D* | 15 | 24 | 6 | 17 | 5 | 13 | 8 | 22 | 17 | 11 | 14 | 14 | 14 |
| | *ID* | 22 | 10 | 5 | 18 | 7 | 8 | 13 | 16 | 18 | 15 | 16 | 6 | 13 |
| | *I* | 12 | 15 | 25 | 12 | 13 | 17 | 14 | 9 | 13 | 20 | 18 | 5 | 14 |
| | *IV* | 11 | 7 | 20 | 10 | 23 | 10 | 15 | 5 | 11 | 9 | 6 | 23 | 13 |
| | *V* | 1 | 5 | 5 | 4 | 13 | 13 | 11 | 9 | 2 | 6 | 7 | 13 | 7 |

TABLE VII

RELATIONSHIP BETWEEN SEED PLUMPNESS AND PLANT TYPE FOR 13 CHARACTERS IN THE $F_2$ OF THE *durum* × *vulgare* CROSS

| Character | Percentage of plants classified as *V* or *IV* | | | Class *C* / Class *A* | Percentage of plants classified as *D* or *ID* | | | Class *C* / Class *A* |
|---|---|---|---|---|---|---|---|---|
| | Class *A* | Class *B* | Class *C* | | Class *A* | Class *B* | Class *C* | |
| Spike form | 16 | 35 | 55 | 3.44 | 54 | 40 | 29 | 0.54 |
| Spike compactness | 26 | 31 | 45 | 1.73 | 67 | 59 | 44 | 0.66 |
| Stem hollowness | 21 | 11 | 24 | 1.14 | 59 | 37 | 40 | 0.68 |
| Stem thickness | 32 | 29 | 53 | 1.66 | 51 | 56 | 40 | 0.78 |
| Rachis width | 39 | 35 | 42 | 1.08 | 41 | 43 | 33 | 0.81 |
| Rachis hairiness | 30 | 38 | 53 | 1.77 | 61 | 57 | 42 | 0.69 |
| Shoulder width | 30 | 31 | 33 | 1.10 | 65 | 62 | 51 | 0.79 |
| Glume l-w ratio | 29 | 36 | 44 | 1.52 | 60 | 51 | 49 | 0.82 |
| Keel sharpness | 30 | 36 | 36 | 1.20 | 57 | 45 | 44 | 0.77 |
| Beak character | 38 | 43 | 44 | 1.16 | 42 | 39 | 35 | 0.83 |
| Keel prominence | 25 | 25 | 33 | 1.32 | 47 | 49 | 42 | 0.89 |
| Brush character | 42 | 47 | 50 | 1.19 | 32 | 42 | 30 | 0.94 |
| Seed character | 29 | 31 | 40 | 1.38 | 63 | 53 | 42 | 0.67 |
| Average | 30 | 33 | 42 | 1.40 | 54 | 50 | 40 | 0.74 |

NOTE.—*The* $\chi^2$ *goodness of fit test applied to* $\frac{\text{Class } C}{\text{Class } A}$ *for % V plus IV versus* $\frac{\text{Class } C}{\text{Class } A}$ *for % D plus ID shows enormous odds that the two sets of ratios are not similar.*

In general, the more shrunken the $F_2$ seed the greater is the *vulgare*-ness of the plants for the characters studied.

The results given in Tables IV, V and VI are more easily analyzed when summarized on a percentage basis. Such a summary is clarified by considering that a plant is *vulgare*-like for a given character if it is recorded as *V* or *IV* for that character and conversely that its inclusion in either of the categories *D* or *ID* designates its resemblance to its 14-chromosome grandparent. The data were therefore summarized on this basis and are shown in Tables VII, VIII and IX.

TABLE VIII

RELATIONSHIP BETWEEN SEED PLUMPNESS AND PLANT TYPE FOR SEVEN CHARACTERS IN THE $F_2$ OF THE *persicum* × *vulgare* CROSS

| Character | Percentage of plants classified as *V* or *IV* | | | Class *C* / Class *A* | Percentage of plants classified as *D* or *ID* | | | Class *C* / Class *A* |
|---|---|---|---|---|---|---|---|---|
| | Class *A* | Class *B* | Class *C* | | Class *A* | Class *B* | Class *C* | |
| Stem thickness | 18 | 22 | 24 | 1.33 | 60 | 67 | 63 | 1.05 |
| Stem hollowness | 8 | 16 | 26 | 3.25 | 76 | 70 | 51 | 0.67 |
| Rachis width | 16 | 26 | 27 | 1.69 | 70 | 51 | 26 | 0.37 |
| Rachis hairiness | 20 | 28 | 29 | 1.45 | 84 | 65 | 45 | 0.54 |
| Shoulder width | 6 | 13 | 8 | 1.33 | 88 | 81 | 82 | 0.93 |
| Glume l-w ratio | 20 | 50 | 39 | 1.95 | 66 | 59 | 59 | 0.89 |
| Beak character | 74 | 68 | 86 | 1.16 | 12 | 19 | 6 | 0.50 |
| Average | 23 | 32 | 34 | 1.48 | 65 | 59 | 47 | 0.72 |

NOTE.—*See footnote of Table VII.*

TABLE IX

RELATIONSHIP BETWEEN SEED PLUMPNESS AND PLANT TYPE FOR 12 CHARACTERS IN THE $F_2$ OF THE *dicoccum* × *vulgare* CROSS

| Character | Percentage of plants classified as *V* or *IV* | | | Class *C* / Class *A* | Percentage of plants classified as *D* or *ID* | | | Class *C* / Class *A* |
|---|---|---|---|---|---|---|---|---|
| | Class *A* | Class *B* | Class *C* | | Class *A* | Class *B* | Class *C* | |
| Spike form | 11 | 10 | 20 | 1.82 | 72 | 73 | 61 | 0.85 |
| Spike compactness | 11 | 16 | 20 | 1.82 | 88 | 73 | 56 | 0.64 |
| Stem hollowness | 7 | 26 | 41 | 5.86 | 72 | 46 | 18 | 0.25 |
| Rachis articulation | 7 | 22 | 23 | 3.29 | 80 | 52 | 57 | 0.71 |
| Rachis width | 15 | 29 | 59 | 3.93 | 65 | 43 | 20 | 0.31 |
| Stem thickness | 13 | 23 | 38 | 2.92 | 57 | 51 | 34 | 0.60 |
| Glume adherence | 43 | 28 | 43 | 1.00 | 30 | 44 | 34 | 1.13 |
| Rachis hairiness | 4 | 19 | 23 | 5.75 | 89 | 69 | 62 | 0.70 |
| Shoulder width | 2 | 4 | 21 | 10.50 | 88 | 81 | 57 | 0.65 |
| Glume shape | 4 | 4 | 25 | 6.25 | 76 | 79 | 43 | 0.57 |
| Keel sharpness | 28 | 21 | 21 | 0.75 | 52 | 59 | 49 | 0.94 |
| Awning | 48 | 44 | 59 | 1.23 | 48 | 37 | 33 | 0.69 |
| Average | 16 | 21 | 33 | 2.06 | 68 | 59 | 44 | 0.65 |

NOTE.—*See footnote of Table VII.*

In the *durum* × *vulgare* cross, Class *C* was more *vulgare*-like than Class *B* in all of the 13 characters and Class *B* was more *vulgare*-like than Class *A* in nine characters. In the *persicum* × *vulgare* cross, Class *C* was more *vulgare*-like than Class *B* in five of the seven characters and Class *B* was more *vulgare*-like than Class *A* in six characters. In the *dicoccum* × *vulgare* cross Class *C* was more *vulgare*-like than Class *B* in 11 of the 12 characters and Class *B* was more *vulgare*-like than Class *A* in 8 of the 12 characters.

The ratio of Class *C* to Class *A* for percentage of *vulgare*-ness (*V* plus *IV*) is shown in the fourth column of Tables VII, VIII and IX. The Class *C* to Class *A* ratio is given in the eighth column for the percentage resemblance to the 14-chromosome parent (*D* plus *ID*). The difference between the two columns of ratios is highly significant in each cross and shows clearly the wide difference in character expression between Classes *A* and *C*.

*Character of Individual Plants with Respect to Classes A, B and C*

The foregoing presentation of the results has been on the basis of population characteristics and tendencies, but it is perhaps even more important to consider the data from the viewpoint of the character of individual plants. This was done in the *durum* × *vulgare* cross by taking *ID* and *D* as indicating *durum*-like character and *IV* and *V* as representing *vulgare*-like character. Category *I*, being a neutral class, was not considered. Plants classified as *D* or *ID* for at least two more characters than as *IV* or *V*, were considered to be *durum*-like. Similarly plants described as *IV* or *V* for at least two more characters than as *D* or *ID*, were called *vulgare*-like. The ratio of *durum*-like to *vulgare*-like was then averaged with the ratio obtained by considering all plants with a majority of *D* and *ID* over *IV* and *V* as *durum*-like, and all those with a majority of *IV* and *V* over *D* and *ID* as *vulgare*-like. The same method was used in the *persicum* × *vulgare* and *dicoccum* × *vulgare* crosses. The relative proportions of plants classified as either *vulgare*-like or like the 28-chromosome parent variety in each plumpness class for each cross are summarized in Table X.

TABLE X

PLANT TYPE RATIOS IN THE THREE INTERSPECIFIC CROSSES

| Plant type ratio | Plumpness classes for $F_2$ seed | | |
|---|---|---|---|
| | *A* | *B* | *C* |
| *persicum* : *vulgare* | 9.3 : 1.0 | 3.1 : 1.0 | 2.2 : 1.0 |
| *durum* : *vulgare* | 2.6 : 1.0 | 1.8 : 1.0 | .88 : 1.0 |
| *dicoccum* : *vulgare* | 12.1 : 1.0 | 5.3 : 1.0 | 2.2 : 1.0 |

The results show that for all three crosses there is a pronounced tendency toward more *vulgare*-ness in Class *B* than in Class *A*, and in Class *C* than in Class *B*. In the *VM* cross, Class *A* is nearly six times as *dicoccum*-like as Class *C* but in the *MP* and *IM* crosses, Class *A* is only four and three times as *persicum*-like and *durum*-like, respectively, as Class *C*. Comparing the results for Class *B* and Class *C*, the slightly shrunken seeds produced on the average about half as many *vulgare*-like plants as did the shrunken seeds. As with field emergence, the results furnish evidence that crosses of *vulgare*

TABLE XI

PROPORTION OF *vulgare*-LIKE PLANTS IN EACH PLUMPNESS CLASS

| Cross | Plumpness classes | | | |
|---|---|---|---|---|
| | *A*, % | *B*, % | *C*, % | Average, % |
| *IM* | 27.8 | 35.7 | 53.2 | 38.9 |
| *MP* | 9.7 | 24.4 | 31.3 | 21.8 |
| *VM* | 7.6 | 15.9 | 31.3 | 18.3 |

with different 28-chromosome wheats do not react alike although certain general tendencies are found in each of them.

The differences as to plant type in the three crosses and the three plumpness classes are seen clearly when the ratios of Table X are given as percentages of *vulgare*-like plants. This is done in Table XI.

*Vulgare-ness of the Vulgare-like Plants in each Plumpness Class*

The *vulgare*-like plants are not equally *vulgare-like* since some are *vulgare*-like in all characters, whereas others may be *vulgare*-like in a bare majority of characters, therefore, it is of interest to examine the character of these plants to determine the relationship between the degree of *vulgare*-ness and the plumpness of the $F_2$ seeds. In Table XII this relationship is revealed for the three crosses. The data on the *vulgare*-like plants are summarized according to the three plumpness classes, and in each case the ratio of *D* plus *ID* to *IV* plus *V* is determined. In the *IM* cross, *vulgare*-like plants of Class *C* were less *vulgare*-like than the *vulgare*-like plants of Classes *B* or *A*. In the *VM* cross the opposite was true. In the *MP* cross the greatest *vulgare*-ness was in the Class *B* *vulgare*-like plants; however the least *vulgare*-ness was in the *vulgare*-like plants of Class *A* as in the *VM* cross. The *IM* results are much at variance with those of the other two crosses and serve to accentuate the differences already noted in the behavior of the three crosses. It is noticeable that the *MP* and *VM* crosses both had very few *vulgare*-like plants in Class *A* and these plants were less *vulgare*-like as groups than any of the other groups shown in Table XII.

TABLE XII

THE RELATIONSHIP BETWEEN $F_2$ SEED PLUMPNESS AND THE DEGREE OF *vulgare*-LIKE CHARACTER IN THE *vulgare*-LIKE $F_2$ PLANTS OF THREE INTERSPECIFIC CROSSES

| Cross | Plumpness class | No. of *vulgare*-like plants | Character categories | | | | | Ratio of "14-chromosome-like" to *vulgare*-like character |
|---|---|---|---|---|---|---|---|---|
| | | | *D* | *ID* | *I* | *IV* | *V* | |
| *IM* | *A* | 28 | 23 | 23 | 67 | 65 | 180 | 1.0 : 5.3 |
| | *B* | 38 | 35 | 42 | 118 | 121 | 174 | 1.0 : 5.1 |
| | *C* | 28 | 26 | 43 | 75 | 93 | 121 | 1.0 : 3.1 |
| *MP* | *A* | 5 | 2 | 6 | 5 | 7 | 15 | 1.0 : 1.8 |
| | *B* | 18 | 11 | 12 | 17 | 27 | 59 | 1.0 : 3.7 |
| | *C* | 17 | 15 | 11 | 19 | 37 | 37 | 1.0 : 2.8 |
| *VM* | *A* | 4 | 10 | 5 | 7 | 12 | 14 | 1.0 : 1.9 |
| | *B* | 15 | 23 | 25 | 46 | 59 | 38 | 1.0 : 2.0 |
| | *C* | 18 | 25 | 20 | 61 | 70 | 56 | 1.0 : 2.8 |

*The Effect of Taking Special Care of Seedlings from Shrunken Seeds*

In the opinion of some breeders, the plants from shrunken seeds in a wide interspecific wheat cross should be given special care. The assumption is that various interspecific character combinations which occur in plants from shrunken seeds, do not occur in plants from the plumper seeds. While the present study has given no evidence that this is true, it was thought worthwhile to see whether or not a population given special seedling care would be more *vulgare*-like than one given ordinary field treatment.

A lot of 150 very shrunken $F_2$ seeds of the cross Vernal emmer × Marquis was divided into two parts, one part being sown shallow in the greenhouse in wooden flats, and the other portion being sown directly in the nursery without any special care. The greenhouse conditions were fairly ideal for the development of wheat seedlings, whereas the nursery conditions were rather severe, the spring being dry and windy, and the nights cold. When the greenhouse plants were well started, they were carefully transplanted to the nursery. The results of the experiment are given in Table XIII.

TABLE XIII

COMPARISON OF PLANTS STARTED IN GREENHOUSE AND IN NURSERY

| Plants started in | No. seeds sown | Stand in June | Emergence, % | Ratio of *dicoccum*-like to *vulgare*-like plants |
|---|---|---|---|---|
| Greenhouse | 75 | 33 | 40.0 | 4.6 : 1.0 |
| Nursery | 75 | 25 | 33.3 | 4.8 : 1.0 |

The results show clearly that the plants from shrunken seeds started in the greenhouse gave approximately the same proportion of *vulgare*-like plants as those sown directly in the field. The stand was better for the greenhouse plants, but this was far outbalanced by the large amount of labor involved in the greenhouse care, and the transplanting. The greenhouse group of plants gave a total of three *vulgare*-like plants. An equal number of *vulgare*-like plants occurred in the nursery-sown group. These two sets of *vulgare*-like plants were about equally *vulgare*-like. The experiment indicated that special care of the shrunken seeds was a waste of time.

## Discussion

It has been proved by Harrington and Smith (3), Harrington (1), McFadden (4), Stevenson (5) and others, that extremely large hybrid populations are necessary if reasonable hope for success in wide wheat crosses is to be entertained. It is of considerable importance, therefore, that suitable methods of dealing with such crosses be developed. The wide variation in the endosperm development of the $F_2$ seeds immediately arrests attention. If the use of any definite portion of these seeds, as segregated on the basis of the degree of "plumpness," is better than the use of all the seeds or of some other part, it

is important to know it. Harrington (2) showed that in a *dicoccum* × *vulgare* cross the shrunken seeds produced a larger proportion of *vulgare*-like plants than did the plumper seeds. The present study has demonstrated the same tendency in two other interspecific crosses.

The establishment of the fact that there is a definite general relationship between the degree of endosperm development of the seed and the morphologic type of the resulting plant raised the question of the closeness of this relationship and its practical implications. The relationship was not found to be close; many plump seeds produced *vulgare*-like plants and many shrunken seeds produced plants resembling the 14-chromosome parent species. While the *vulgare*-like plants from shrunken seeds were distinctly more *vulgare*-like than those from plump seeds, the latter were found to be as *vulgare*-like as many of those from shrunken seeds. Furthermore, the shrunken seeds that were coaxed to grow under ideal conditions produced plants that were no more *vulgare*-like than those from shrunken seeds sown directly in the field nursery.

A study of the plants from each seed plumpness class of each cross revealed a wide assortment of character combinations. There was every indication that *vulgare* plants with desired character resemblances to the 14-chromosome parent would be obtainable from any of the plumpness classes, providing the population was very large.

The general conclusions from the breeding standpoint are that no attention need be paid to the endosperm development of the $F_2$ seeds, for while plants from the shrunken seeds are more *vulgare*-like than those from the other seeds, the shrunken seeds make up less than a third of the whole population and do not yield character combinations unobtainable from plants from plump seeds. However, examination of the results from the different crosses indicates that each cross must be considered by itself. The *durum* × *vulgare* cross *(IM)* was relatively high in proportion of shrunken seeds, relatively low in germination of plump seeds and relatively high in the total proportion of *vulgare*-like plants as well as in the proportion of *vulgare*-like plants from plump seeds. In this cross, there would appear to be no good reason for paying attention to the plumpness of the $F_2$ seeds.

The *persicum* × *vulgare* cross *(MP)* was (like *IM*) distinctly high in proportion of shrunken seeds, high also (unlike *IM*) in the germination of the plump seeds, low compared to *IM* in the total proportion of *vulgare*-like plants and very low in the proportion of *vulgare*-like plants from plump seeds. In this cross, the high proportion of shrunken and slightly shrunken seeds and the large proportion of *vulgare*-like plants from these two seed classes seem to favor the paying of special attention to the shrunken $F_2$ seeds. The high germination of the plump seeds is an offsetting factor. On the whole, in a cross with this behavior, it might be advisable to classify the $F_2$ seeds into two groups, the plump and shrunken, and sow only the latter group.

The *dicoccum* × *vulgare* cross *(VM)* studied by Harrington (2) was very low in proportion of shrunken seeds and higher than either of the other

crosses in percentage germination, using the germination of the 14-chromosome check varieties as a standard, but in proportion of *vulgare*-like plants this cross was even lower than *MP*, particularly in Class *B*. The results suggest that in a cross of this kind the large proportion of plump seeds is offset by its low degree of *vulgare*-ness and the low proportion of shrunken seeds is offset by its fairly high degree of *vulgare*-ness. On the whole then, there seems to be little reason for paying attention to $F_2$ seed plumpness in this cross.

No doubt the use of other species would reveal additional differences among interspecific crosses. Various investigators have shown that wide genetic differences exist between different wheats of the same chromosome number. A comprehensive understanding of the behavior of crosses between 14- and 21-chromosome wheats would necessitate a vast amount of work involving at least a dozen representatives of each chromosome group. Therefore, in a limited study, it is necessary to restrict the conclusions specifically to the material used. However, the present work is sufficiently wide in its scope to suggest a general procedure that is not likely to be far wrong, *viz.*, the use of a *large* population of $F_2$ seeds sown with ordinary care in a field nursery.

## Summary

1. A study was made of the relationship between endosperm development and plant type in the $F_2$ generation of three interspecific wheat crosses: Iumillo *(durum)* × Marquis *(vulgare)*, Marquis × Black Persian *(persicum)* and Vernal *(dicoccum)* × Marquis, the latter from data published in 1931 (2).

2. The $F_2$ seeds of each cross were divided for convenience into three groups on the basis of seed plumpness, *viz.*, plump (Class *A*), slightly shrunken (Class *B*) and shrunken (Class *C*). The proportions of seeds in the three classes when averaged for the three crosses were 35, 37 and 28%.

3. Seed plumpness proved to be closely related to seedling emergence.

4. In each of the crosses, plants from plump seeds were less *vulgare*-like than those from slightly shrunken seeds and the latter were less *vulgare*-like than plants from shrunken seeds.

5. Each of the crosses showed distinctive behavior as follows: the Iumillo × Marquis cross *(IM)* resembled the Marquis × Black Persian cross *(MP)* in the distribution of seeds according to the three plumpness classes. In seedling emergence the *IM* cross was very low in Class *A*, whereas the *MP* cross was high and the Vernal × Marquis cross *(VM)* was fairly high. In the $F_2$ distribution for each of the morphologic characters, the *IM* cross differed distinctly from the other two by showing a greater proportion of *vulgare*-ness in each class. Consideration of the type of each $F_2$ plant pointed to a greater similarity between the *MP* and *VM* crosses than between either of these and the *IM* cross. The *MP* cross differed from *VM* in that the latter showed much less *vulgare*-ness in Class *A*.

6. From the standpoint of breeding for *vulgare*-like hybrids possessing only one or two characters of the 14-chromosome species, the results indicated that the plumper half of the $F_2$ seeds of the *persicum* × *vulgare* cross could well be discarded in favor of using the more shrunken half, but in the case of the *durum* × *vulgare* and *dicoccum* × *vulgare* crosses, the best procedure would be to sow the $F_2$ seeds without regard to their plumpness.

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# CHIASMA FREQUENCY IN SPECIES AND SPECIES HYBRIDS OF *AVENA*[1]

By Jane D. Spier[2]


## Abstract

(i) Chiasma frequency at metaphase has been studied in the following species and species hybrids: *A. strigosa* Schreb.; *A. brevis* Roth; *A. Wiestii* Steud.; *A. barbata* Pott; *A. abyssinica* Hochst. (2 strains); *A. sterilis* L. (2 strains); *A. sativa* L. var. Radnorshire Sprig; a triploid hybrid, *A. barbata* × *A. strigosa;* a pentaploid hybrid, *A. abyssinica* "naine" × *A. sterilis* maxima; and a hexaploid hybrid, *A. sterilis* (white) × *A. sativa* var. Radnorshire Sprig.

(ii) The various factors that may affect chiasma frequency are discussed and it is concluded that there may be some parallelism between closeness of relationship and similarity in chiasma formation, as genetical control is one factor affecting the process. The results are examined in the light of this conclusion.

(iii) The homogeneity of species in respect to proportion of bivalents with zero, one, two, three, or four chiasmata is tested by means of $\chi^2$. The test indicates no significant difference between the closely related diploid species, *A. brevis* and *A. strigosa*, but both differ significantly from the less closely related *A. Wiestii*. The differences between the tetraploid species are of questionable significance, those between the hexaploids, insignificant. It is suggested that the lack of significant differences among these last-mentioned forms may be due to a multiplication, in the polyploid, of genes favoring high chiasma frequency. In this way a maximum effect might be brought about, and interspecific differences with respect to this character would consequently be lacking.

(iv) High chiasma frequency in the hybrid might be regarded as strong evidence of homology, and consequently of relationship between parental forms. The triploid hybrid, *A. barbata* × *A. strigosa* shows a high chiasma frequency at metaphase, a condition consistent with the close relationship of the parental forms. The pentaploid hybrid, as might be expected, has a low chiasma frequency. The chiasma frequency of the hexaploid hybrid, *A. sterilis* × *A. sativa* var. Radnorshire Sprig does not differ significantly from that of its low-frequency parent, *A. sterilis*. Cytological observations in this hybrid, therefore, reveal only a slight degree of non-homology between parental chromosomes. It is noted that there are certain characters of *A. sativa* var. Radnorshire Sprig that suggest affinity with *A. byzantina*, a species commonly associated with *A. sterilis* in relationship schemes.


The interspecific relationships that exist within the section *Euavena* are clearly indicated by the morphological characteristics of the different species and by serological reactions between them. The genealogical tree of Thellung (17) shows these interspecific relationships in a generally accepted form.

Nishiyama (12) made a cytological study of various interspecific hybrids of *Avena*. He expressed the degree of relationship between parental species by the ratio in the hybrid of:

$$\frac{\text{number of bivalents found}}{\text{number of bivalents expected}}.$$

A full affinity between parental species is indicated by a value of one.

According to Nishiyama (12), those species which are closely related in the genealogical tree of Thellung (17) showed cytological relationships approaching unity, while more distantly related species gave, on the whole, cytological relationships that were considerably lower than one.

[1] *Manuscript received May 22, 1934.*
*Contribution from the Department of Botany, McGill University, Montreal, Quebec.*
[2] *Lecturer, Department of Botany, McGill University.*

It is difficult to determine the degree of homology that exists between two chromosomes. For many organisms, it is best estimated at present by comparative studies of chiasma frequency. As Darlington (3) points out, there are difficulties in the way of such a procedure. Not only homology, but also the arrangement of homologous parts is involved. Moreover, competition in pairing in a polyploid may result in lowered chiasma frequency, and it seems evident that environmental or developmental conditions may affect prophase pairing or chiasma formation (Kihara (10), Hollingshead (7) ).

Nishiyama's (12) findings are based upon a rough approximation to chiasma frequency, but his method reveals only gross differences in the species hybrids. It was decided, therefore, to study chiasma frequency at metaphase of various species and interspecific hybrids of *Avena* in order to detect, if possible, any significant differences with respect to this characteristic.

## Material and Methods

The species and species hybrids shown in Table I were studied.

TABLE I

SPECIES AND SPECIES HYBRIDS STUDIED

| No. | Species | Chrom. No. (2*N*) | Origin | Year grown |
|---|---|---|---|---|
| 1 | *A. brevis* Roth | 14 | Prof. Fraser, Cornell Univ., Ithaca, N.Y. | 1932 |
| 2 | *A. Wiestii* Steud. | 14 | Dr. T. R. Stanton, U.S.D.A., C.I. No. 1994. | 1932 |
| 3 | *A. strigosa* Schreb. | 14 | From Spain, via Bureau of App. Bot. and Plant Breeding, U.S.S.R. | 1932 |
| 4 | *A. barbata* Pott | 28 | Dr. T. R. Stanton, U.S.D.A., C.I. No. 2467. | 1932 |
| 5 | *A. abyssinica* Hochst. ("naine") | 28 | Ch. Crepin, Clermont, Ferrand. Originally found by Vilmorin. | 1932 |
| 6 | *A. abyssinica* Hochst. (gray) | 28 | Dr. T. R. Stanton, U.S.D.A., C.I. No. 2109–6. | 1930 |
| 7 | *A. sterilis* L. (maxima) | 42 | H. Hunter, School of Agriculture, Cambridge. | 1932 |
| 8 | *A. sterilis* L. (white) | 42 | Axel Pedersen, Agr. H. School, Copenhagen. | 1932 |
| 9 | *A. sativa* L. var. (Radnorshire Sprig) | 42 | E. T. Jones, Aberystwyth. | 1932 |
| 10 | 3$x$ hybrid (No. 4 × No. 3) | 21 | | 1929 |
| 11 | 5$x$ hybrid (No. 5 × No. 7) | 35 | | 1929 |
| 12 | 6$x$ hybrid (No. 8 × No. 9) | 42 | | 1930 |

According to Emme (4), the question of the chromosome number of the true *A. Wiestii* Steudel is as yet unsettled. Huskins (8) reports that the haploid chromosome number of this species is seven. Emme (4) remarks that as he gives no information on distribution or specific characteristics of this wild form, she is uncertain of its identity. The form investigated by Huskins (8) is the *A. Wiestii* Steudel of this paper. It is described by Stanton and Dorsey (16).

The species hybrids and *A. abyssinica* (gray) were grown at the John Innes Horticultural Institute, and the remaining pure species at Macdonald College, P.Q.

Permanent aceto-carmine smears were made of microspore mother cells of all material except the triploid hybrid. The triploid hybrid stamens were fixed in Kihara's Carnoy-Flemming, embedded in paraffin and sectioned at 14 $\mu$. The sections were stained by Newton's iodine crystal violet method. Metaphase plates of the first maturation division were studied and the number of chiasmata counted in cells whose full chromosome complement could clearly be distinguished.

## Observations

Tables II to V show the number of bivalents per cell with zero, one, two, three or four chiasmata in diploid, tetraploid and hexaploid species and in triploid, pentaploid and hexaploid hybrids.

*(a) Diploid Species*

Chiasma frequencies of *A. brevis* and *A. strigosa* are very similar (Table II). The average number of chiasmata per cell in 20 cells is 15.30 in the former, 15.15 in the latter, and in both species the proportions of bivalents with zero, one, two, or three chiasmata are alike.

TABLE II

CHIASMA FREQUENCY IN 2*x* SPECIES OF *Avena*

| Cell No. | Chiasmata per bivalent per cell | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. strigosa* | | | | *A. Wiestii* | | | | *A. brevis* | | | |
| | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1 | — | 1 | 3 | 3 | — | — | 5 | 2 | — | — | 3 | 4 |
| 2 | — | — | 4 | 3 | — | — | 6 | 1 | — | 1 | 5 | 1 |
| 3 | — | 2 | 4 | 1 | — | — | 7 | — | — | — | 5 | 2 |
| 4 | — | 1 | 5 | 1 | — | — | 6 | 1 | — | 1 | 3 | 3 |
| 5 | — | 1 | 4 | 2 | — | 1 | 6 | — | — | 1 | 6 | — |
| 6 | — | 1 | 5 | 1 | — | 1 | 4 | 2 | — | — | 6 | 1 |
| 7 | — | 1 | 3 | 3 | — | 1 | 6 | — | 1 | 1 | 2 | 3 |
| 8 | — | 1 | 5 | 1 | — | 2 | 5 | — | — | — | 4 | 3 |
| 9 | — | 2 | 2 | 3 | — | 1 | 6 | — | — | 2 | 5 | — |
| 10 | — | 1 | 4 | 2 | — | 2 | 5 | — | — | — | 3 | 4 |
| 11 | — | — | 5 | 2 | — | 2 | 5 | — | — | 1 | 4 | 2 |
| 12 | — | 2 | 2 | 3 | — | 1 | 6 | — | — | 3 | 2 | 2 |
| 13 | — | — | 6 | 1 | — | 2 | 5 | — | — | — | 6 | 1 |
| 14 | — | 1 | 4 | 2 | — | 2 | 5 | — | — | 2 | 4 | 1 |
| 15 | — | — | 4 | 3 | — | 1 | 6 | — | — | — | 6 | 1 |
| 16 | — | — | 6 | 1 | — | 1 | 5 | 1 | — | 1 | 3 | 3 |
| 17 | — | — | 5 | 2 | — | 1 | 6 | — | — | — | 4 | 3 |
| 18 | — | 1 | 4 | 2 | — | 1 | 6 | — | — | — | 5 | 2 |
| 19 | 1 | — | 5 | 1 | — | 1 | 6 | — | — | — | 5 | 2 |
| 20 | — | — | 4 | 3 | — | 2 | 5 | — | — | 1 | 2 | 4 |
| Total | 1 | 15 | 84 | 40 | 0 | 22 | 111 | 7 | 1 | 14 | 83 | 42 |
| Average per cell | .05 | .75 | 4.2 | 2.0 | 0 | 1.1 | 5.55 | .35 | .05 | .70 | 4.15 | 2.1 |
| Mean no. chiasmata per cell | 15.15 ± 0.24 | | | | 13.25 ± 0.24 | | | | 15.30 ± 0.24 | | | |

TABLE III

CHIASMA FREQUENCY IN 4x SPECIES OF *Avena*

| Cell No. | Chiasmata per bivalent per cell | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. barbata* | | | | *A. abyssinica* ("naine") | | | | *A. abyssinica* (gray) | | | |
| | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1 | — | — | 11 | 3 | — | 3 | 10 | 1 | — | 2 | 11 | 1 |
| 2 | — | — | 12 | 2 | 1 | 1 | 10 | 2 | — | 2 | 10 | 2 |
| 3 | — | 2 | 8 | 4 | — | 5 | 5 | 4 | — | 4 | 9 | 1 |
| 4 | — | — | 12 | 2 | — | 4 | 8 | 2 | — | 1 | 8 | 5 |
| 5 | — | — | 12 | 2 | — | 4 | 9 | 1 | — | 2 | 9 | 3 |
| 6 | — | 4 | 6 | 4 | — | 3 | 11 | — | — | 1 | 12 | 1 |
| 7 | — | 4 | 7 | 3 | — | 3 | 7 | 4 | — | 4 | 8 | 2 |
| 8 | — | — | — | — | — | 4 | 9 | 1 | — | 2 | 12 | — |
| 9 | — | — | — | — | — | 3 | 9 | 2 | — | 1 | 11 | 2 |
| 10 | — | — | — | — | — | 3 | 11 | — | — | 2 | 9 | 3 |
| 11 | — | — | — | — | — | 3 | 10 | 1 | — | 4 | 9 | 1 |
| 12 | — | — | — | — | 1 | 2 | 9 | 2 | — | 4 | 9 | 1 |
| 13 | — | — | — | — | — | 4 | 8 | 2 | — | — | 13 | 1 |
| Total | — | 10 | 68 | 20 | 2 | 42 | 116 | 22 | — | 29 | 130 | 23 |
| Average per cell | — | 1.43 | 9.71 | 2.86 | .15 | 3.23 | 8.93 | 1.69 | — | 2.23 | 10.0 | 1.77 |
| Mean no. chiasmata per cell | 29.42 ± 0.40 | | | | 26.15 ± 0.30 | | | | 27.54 ± 0.30 | | | |

TABLE IV

CHIASMA FREQUENCY IN 6x SPECIES OF *Avena*

| Cell No. | Chiasmata per bivalent per cell | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. sterilis* 5*n* parent | | | | *A. sterilis* 6*n* parent | | | | *A. sativa*, var. Radnorshire Sprig | | | |
| | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1 | — | 1 | 13 | 7 | — | 1 | 13 | 7 | — | 1 | 9 | 11 |
| 2 | — | 2 | 11 | 8 | — | 1 | 11 | 9 | — | 3 | 10 | 8 |
| 3 | — | 3 | 8 | 10 | — | 2 | 12 | 7 | — | — | 17 | 4 |
| 4 | — | 3 | 12 | 6 | — | 3 | 14 | 4 | — | — | 14 | 7 |
| 5 | — | 1 | 11 | 9 | — | 3 | 14 | 4 | — | 1 | 13 | 7 |
| 6 | — | — | 14 | 7 | — | 3 | 11 | 7 | — | — | 16 | 5 |
| 7 | — | 2 | 14 | 5 | — | 1 | 16 | 4 | — | — | 14 | 7 |
| 8 | — | 2 | 14 | 5 | — | — | — | — | — | — | — | — |
| 9 | — | 1 | 11 | 9 | — | — | — | — | — | — | — | — |
| Total | — | 15 | 108 | 66 | — | 14 | 91 | 42 | — | 5 | 93 | 49 |
| Average per cell | — | 1.65 | 12.0 | 7.33 | — | 2 | 13 | 6 | — | .71 | 13.29 | 7.00 |

TABLE V

CHIASMA FREQUENCY IN 3x*, 5x AND 6x SPECIES HYBRIDS, *Avena*

| Cell No. | Chiasmata per bivalent per cell | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6x hybrid | | | | 5x hybrid | | | | 3x hybrid | | | | |
| | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | 4 |
| 1 | — | 2 | 14 | 5 | 8.5 | 9 | — | — | 3.0 | 1 | .5 | 5 | 1 |
| 2 | — | 1 | 17 | 3 | 9.5 | 7 | 1 | — | 2.5 | 2 | — | 6 | — |
| 3 | — | 5 | 11 | 5 | 10.5 | 7 | — | — | 2.5 | 3 | 1 | 4 | — |
| 4 | — | 4 | 10 | 7 | 7.5 | 10 | — | — | 2.5 | 1 | 3 | 3 | 1 |
| 5 | — | 2 | 13 | 6 | 7.5 | 10 | — | — | 3.5 | — | 2 | 5 | — |
| 6 | — | 2 | 12 | 7 | 10.5 | 6 | 1 | — | 3.5 | 1 | 2 | 4 | — |
| 7 | — | 1 | 16 | 4 | 9.5 | 6 | 2 | — | 3.5 | 1 | 5 | 1 | — |
| 8 | — | 1 | 13 | 7 | 8.5 | 8 | 1 | — | 2.5 | 1 | 3 | 4 | — |
| 9 | — | 2 | 12 | 7 | 8.5 | 8 | 1 | — | 3.5 | — | 3 | 4 | — |
| 10 | — | 3 | 11 | 7 | 9.5 | 7 | 1 | — | 3.5 | 2 | 2 | 3 | — |
| Total bivalent | — | 23 | 129 | 58 | 90.0 | 78 | 7 | — | 30.5 | 12 | 21.5 | 39 | 2 |
| Average per cell | — | 2.3 | 12.9 | 5.8 | 9 | 7.8 | .7 | — | 3.05 | 1.2 | 2.15 | 3.9 | .2 |
| % per cell (hybrids) | — | 11 | 61 | 28 | 51.5 | 44.5 | 4.0 | — | 29 | 11 | 21 | 37 | 2 |
| % per cell (♂ parent) | — | 4 | 63 | 33 | — | 8 | 57 | 35 | — | 11 | 60 | 28 | — |
| % per cell (♀ parent) | — | 9 | 62 | 29 | 1 | 22 | 64 | 13 | 1 | 10 | 70 | 20 | — |

* *A. sterilis (white)* × *A. sativa var. Radnorshire Sprig, 6x hybrid; A. abyssinica ("naine")* × *A. sterilis maxima, 5x hybrid; A. barbata* × *A. strigosa, 3x hybrid.*

*A. Wiestii* shows a lower average per cell (13.25 chiasmata) for 20 cells, and the proportion of bivalents with three chiasmata is definitely lower than in either *A. brevis* or *A. strigosa*. The unpaired chromosomes noted in *A. brevis* and *A. strigosa* were the result of anaphase separation of previously paired homologous chromosomes. As anaphase separation was not evident in any of the cells of *A. Wiestii*, the latter material was fixed at an obviously slightly earlier stage, and the difference between *A. Wiestii* and the other species is thereby emphasized.

Testing the independence of different species in respect to chiasma formation (Table VI), it is clear that the difference between *A. Wiestii* and *A. brevis* or *A. strigosa* is statistically significant while that between *A. brevis* and *A. strigosa* is not. Figs. 1, 2 and 3 show the chromosome complements of these three diploid species.

FIGS. 1–5. *Metaphase stage of first maturation division. Figures beside bivalents indicate numbers of chiasmata. Fig. 1. A. strigosa Schreb.; Fig. 2. A. brevis Roth.; Fig. 3. A. Wiestii Steudel.; Fig. 4. A. barbata Pott.; Fig. 5. A. abyssinica Hochst. ("naine"). Magnification* × *1300.*

TABLE VI

HOMOGENEITY OF SPECIES IN RESPECT TO CHIASMA FORMATION, $\chi^2$ TEST

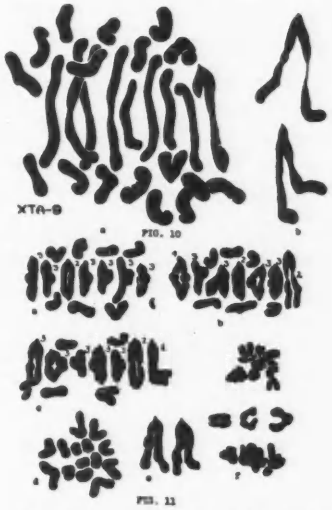
| Species | Chiasmata | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | Total bivalents | $\chi^2$ | D.F. | Limit for significance ($\chi^2$ for $p = .05$) |
| *A. Wiestii* | 0 | 22 | 111 | 7 | 140 | 31 | 3 | 7.8 |
| *A. brevis* | 1 | 14 | 83 | 42 | 140 | | | |
| *A. strigosa* | 1 | 15 | 84 | 40 | 140 | .09 | 3 | 7.8 |
| *A. brevis* | 1 | 14 | 83 | 42 | 140 | | | |
| *A. Wiestii* | 0 | 22 | 111 | 7 | 140 | 29 | 3 | 7.8 |
| *A. strigosa* | 1 | 15 | 84 | 40 | 140 | | | |
| *A. abyssinica* ("naine") | 2 | 42 | 116 | 22 | 182 | 10 | 3 | 7.8 |
| *A. barbata* | 0 | 10 | 68 | 20 | 98 | | | |
| *A. abyssinica* ("naine") | 2 | 42 | 116 | 22 | 182 | 5.1 | 3 | 7.8 |
| *A. abyssinica* (gray) | 0 | 29 | 130 | 23 | 182 | | | |
| *A. barbata* | 0 | 10 | 68 | 20 | 98 | 4.0 | 2 | 5.9 |
| *A. abyssinica* (gray) | 0 | 29 | 130 | 23 | 182 | | | |
| *A. sativa* | 0 | 5 | 93 | 49 | 147 | 4.8 | 2 | 5.9 |
| *A. sterilis* (6) | 0 | 14 | 91 | 42 | 147 | | | |
| *A. sterilis* (6) | 0 | 14 | 91 | 42 | 147 | 1.5 | 2 | 5.9 |
| *A. sterilis* (5) | 0 | 15 | 108 | 66 | 189 | | | |
| *A. sterilis* (5) | 0 | 15 | 108 | 66 | 189 | 3.1 | 2 | 5.9 |
| *A. sativa* | 0 | 5 | 93 | 49 | 147 | | | |
| *A. barbata* | 0 | 10 | 68 | 20 | 98 | 3.1 | 3 | 7.8 |
| *A. strigosa* | 1 | 15 | 84 | 40 | 140 | | | |
| *A. Wiestii* | 0 | 22 | 111 | 7 | 140 | 5.6 | 2 | 5.9 |
| *A. abyssinica* (gray) | 0 | 29 | 130 | 23 | 182 | | | |
| *A. Wiestii* | 0 | 22 | 111 | 7 | 140 | 14.1 | 2 | 5.9 |
| *A. barbata* | 0 | 10 | 68 | 20 | 98 | | | |
| *6x* hybrid | 0 | 23 | 129 | 58 | 210 | .20 | 2 | 5.9 |
| *A. sterilis* (6) | 0 | 14 | 91 | 42 | 147 | | | |
| 6x hybrid | 0 | 23 | 129 | 58 | 210 | 7.2 | 2 | 5.9 |
| *A. sativa* | 0 | 5 | 93 | 49 | 147 | | | |

*(b) Tetraploid Species*

Chiasma frequencies for tetraploid species are recorded in Table III. Applying the $\chi^2$ test to the totals from Table III, the difference between chiasma frequency in *A. barbata* and *A. abyssinica* "naine" is found to be statistically significant. The difference between the two forms of *A. abyssinica* and that between *A. barbata* and *A. abyssinica* (gray) are not statistically significant. As the number of tetraploid cells is low, and as the mean number of chiasmata per cell appears to differ, a comparison between mean chiasma frequencies of the different species is also made. In order to increase the

number of observations upon which the estimates of the standard errors are based, both diploid and tetraploid figures are included in an analysis of variance (6). The standard deviation thus calculated is 1.075. Table VII shows significant differences in mean chiasma frequency per cell.

FIGS. 6–9. *First meiotic division. Fig. 6. A. sterilis L (parent of 5x hybrid); Fig. 7. A. sterilis L (parent of 6x hybrid); Fig. 8. A. sativa L var. Radnorshire Sprig; Fig. 9. 6x hybrid: a. 21 bivalents of one spore mother cell; b. quadrivalent configurations. Magnification × 1300.*



FIGS. 10 and 11. *First meiotic division. Fig. 10. 5x hybrid: a. 9 bivalents, 17 univalents of one cell; b. trivalent configurations. Fig. 11. 3x hybrid: a. 7 univalents, 7 bivalents; b. 6 univalents, 6 bivalents, 1 trivalent; c. 5 univalents, 5 bivalents, 2 trivalents; d. polar view showing peripheral position of the 7 univalents; e. typical trivalents with one chromosome showing two chiasmata in one arm, and one chiasma in the other arm; f. equational division of lagging univalents. Magnification × 1300.*

TABLE VII

SIGNIFICANT DIFFERENCES IN MEAN CHIASMA FREQUENCY PER CELL IN VARIOUS SPECIES OF *Avena*

| Species | Mean number chiasmata per cell | $n$ | Standard error of mean | Mean difference | Standard error, $M_1-M_2$ | Necessary difference between means† |
|---|---|---|---|---|---|---|
| (1)**A. strigosa* | 15.15 | 20 | .24038 | 1.9 (1–2) | .3399 | .66620 |
| (2) *A. Wiestii* | 13.25 | 20 | .24038 | .15 (1–3) | .3399 | .66620 |
| (3) *A. brevis* | 15.30 | 20 | .24038 | 2.05 (2–3) | .3399 | .66620 |
| (4) *A. barbata* | 29.42 | 7 | .40627 | 3.27 (4–5) | .5040 | .98784 |
| (5) *A. abyssinica* "naine" | 26.15 | 13 | .29816 | 1.88 (4–6) | .5040 | .98784 |
| (6) *A. abyssinica* (gray) | 27.54 | 13 | .29816 | 1.39 (5–6) | .4217 | .82653 |

* *The meaning of these numbers will be clear from Column 5, in which pairs of "mean numbers Xta per cell" obtained from Column 2, and identified by the numbers in brackets (obtained from Column 1), are subtracted from each other, and the results tabulated.*

† *Necessary difference = standard error of mean difference* × $t_{(p=.05,\ n=60)}$

The only mean difference that is insignificant is that between *A. strigosa* and *A. brevis*.

Fig. 4 illustrates the chromosome complement of *A. barbata*, Fig. 5 that of *A. abyssinica* "naine". In the latter there are two unpaired chromosomes.

*(c) Hexaploid Species*

Chiasma frequencies of hexaploid species are given in Table V. The $\chi^2$ test (Table VI) fails to reveal any significant differences between chiasma frequencies of the species investigated. Figs. 6, 7 and 8 show metaphase stages of the hexaploid forms.

*(d) 6x Hybrid*

There is little difference between chiasma formation in the hexaploid hybrid (Fig. 9*a*) and in its low-frequency parent, *A. sterilis*. The difference between the hybrid and its other parent, *A. sativa* var. Radnorshire Sprig, is statistically significant. (See Table VI.) Multivalent configurations were seen frequently. Two out of ten cells selected at random showed quadrivalent complexes (Fig. 9*b*).

*(e) 5x Hybrid*

Chiasma frequency in the hybrid is markedly lower than in its parents, *A. abyssinica* "naine" and *A. sterilis*. With complete allosynapsis, and no autosynapsis, seven univalent chromosomes would occur at the first maturation division. In the hybrid, the number of univalent chromosomes varied from 13 to 23 (Fig. 10*a*). In 30 cells selected at random, six trivalent configurations were seen (Fig. 10*b*): no quadrivalents were found.

*(f) 3x Hybrid*

Pairing was closer in the triploid hybrid, *A. barbata* × *A. strigosa*, than in the pentaploid hybrid. In spite of the fact that at least five of the seven supernumerary chromosomes of *A. barbata* remained unpaired in all of the observed cells (Figs. 11*a*, *b*, *c*, *d*), the average number of chiasmata per bivalent of the hybrid was quite high, being 1.72, while those of its parents are 2.10 and 2.16 respectively. Two of twelve cells showed multivalent configurations. One had one, the other two, trivalents.

## Discussion

In most organisms, the pairing of chromosomes at metaphase of the first division of meiosis is conditioned by chiasma formation which takes place between paired homologous chromosomes during pachytene. The zygotene synapsis depends primarily upon the degree of homology which exists between similar paternal and maternal chromosomes, but the following factors may have an effect, more or less marked, either upon prophase pairing or upon subsequent chiasma formation:

(*a*) While pairing is primarily conditioned by homology, it also depends upon the arrangement of homologous parts, and an inversion may interfere with pairing in a heterozygous individual while it causes no external varietal or specific differentiation between the parents.

(*b*) The amount of pairing may vary at different stages in anther development. Hollingshead (7) has shown that when the "general stage" of the anther was late, that is, when most of the cells of the anther had completed division, the proportion of univalents at meiosis was higher than normal in a number of Marquis wheat plants.

(*c*) It is possible that external conditions may influence pairing as Kihara has suggested (10).

(*d*) Competition in pairing may cause a reduction in chiasma frequency in polyploid forms.

(*e*) The pairing process is subject to genetical control. Pairing of homologous chromosomes may take place normally at early stages, yet through failure of chiasma formation there may, at metaphase, be no indication of this earlier association. This condition is seen in an asynaptic maize, where it is shown to be due to an "asynaptic gene" (Beadle (2) ). Genetic factors may also interfere with the early association. The "*c*" chromosome missing from certain asynaptic 40-chromosome dwarf fatuoid oats and speltoid wheats apparently carries factors necessary for normal pairing (Huskins and Hearne (9) ). Peto (14) investigated a number of hybrids between two different genera of the Gramineae, *Festuca* and *Lolium*. On back crossing a number of individuals of a given $F_1$ population to one parent, the progeny fell into markedly different classes with respect to chiasma frequency. This fact is attributed to segregation of genetical factors limiting chiasma formation.

It is very evident from these and similar observations that regularity in pairing depends not only upon homology, but also upon environmental and developmental influences, and upon a balanced condition of genes, or the presence of definite genes, that permit prophase pairing of similar chromosomes, and the maintenance up to anaphase of this pairing through chiasma formation.

*Comparison Between Chiasma Frequencies of Different Species*

Any significant difference in chiasma frequency of the oats species under consideration in this paper may conceivably be dependent upon one or all of the above mentioned factors. The chances of structural hybridity must, however, be small in the individuals of a self-fertilizing genus such as *Avena*, and the developmental effect was probably eliminated in these experiments. Fixations were made at what appeared to be comparable stages in anther development, that is when the majority of cells were at metaphase stage, and, as already noted, the various oats species, with the exception of *A. abyssinica* (gray), were grown simultaneously under similar environmental conditions in experimental plots. In so far, then, as the differences noted are probably due chiefly to differences in the genes which affect pairing and chiasma formation, we might expect some parallelism between similarity in chiasma formation and closeness of relationship. But the use of chiasma frequency as an indication of specific relationships is seriously limited by the fact that gene differences leading to wide variation in respect to this characteristic may occur within a pure species (Beadle (2), Huskins and Hearne

(9), Philp and Huskins (15), Armstrong and Huskins (1) ). Hence a difference in chiasma frequency between two species does not *necessarily* imply that the two species concerned are not closely related. It seems more likely, however, that in a marked similarity of chiasma frequencies of two different species we have evidence in favor of a close relationship. At most, we may expect some parallelism between close relationship and similarity in chiasma formation.

We may review the present results in relation to the genealogical scheme of Thellung (17) which is given in Table VIII in a slightly modified form.

TABLE VIII

GENEALOGICAL SCHEME OF THELLUNG (17)

| | | | | |
|---|---|---|---|---|
| Kulturformen | *A. byzantina* C. Koch | *A. nuda* L. ? *A. sativa* L. | *A. strigosa* Schreber (inkl. *A. brevis* Roth) | *A. abyssinica* Hochst. |
| Wildformen | 1. *A. sterilis* L. | 2. *A. fatua* L. | 3. *A. barbata* Pott ex Link | 3a. *A. Wiestii* Steudel |
| | § 1. Biformes | § 2. Conformes | | |

The species, arranged according to mean chiasma frequency per bivalent, give the following series: *A. abyssinica* "naine", 1.87; *A. Wiestii*, 1.89; *A. abyssinica* (gray), 1.96; *A. barbata*, 2.10; *A. strigosa*, 2.16; *A. brevis*, 2.18; *A. sterilis*, 2.19; *A. sterilis*, 2.26; *A. sativa* var. Radnorshire Sprig, 2.28. As the differences are slight, the variability within species wide, and the number of cells upon which the mean is based is small in some cases, one cannot attach much importance to these figures. However, the relative positions of the different species in the series are suggestive enough to warrant the application of statistical methods to the data available.

The homogeneity of species in respect to frequency of bivalents with zero, one, two, three, or four chiasmata is tested by means of $\chi^2$. No significant difference is found between the three related species *A. brevis*, *A. strigosa* and *A. barbata*. *A. Wiestii*, which has a relatively low chiasma frequency, differs significantly from the other diploid species and from *A. barbata*, but does not differ significantly from the related species *A. abyssinica* (gray). So far, the results are consistent with the idea that similarity in chiasma formation may be correlated with closeness of relationship. However, the results within the tetraploid and hexaploid groups fail to reveal the expected parallelism.

Among the tetraploids, *A. barbata* and *A. abyssinica* (gray) show no significant difference although they fall into different subdivisions of Thellung's (17) table. There is a significant difference between *A. barbata* and *A. abyssinica* "naine", but as the latter had unpaired univalents in two out of thirteen cells investigated, and as it was slightly dwarf in habit, it is probably

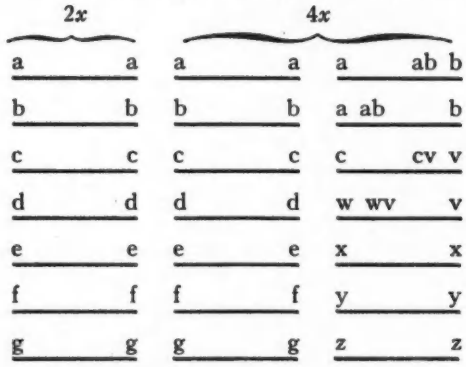
an aberrant form, and *A. abyssinica* (gray) is the more typical representative of the species. The mean chiasma frequency per cell of *A. barbata* did differ significantly from both strains of *A. abyssinica*, but as the two strains also differed significantly from each other, it is obvious that intraspecific differences may be quite as significant as interspecific ones. *A. sativa* and *A. sterilis* fall into different sections of Thellung's (17) table, but the $\chi^2$ test fails to reveal any significant difference between any of the hexaploid forms studied. It is possible that a concentration of genes favoring chiasma formation is responsible in these polyploids for a maximum effect, thus preventing cytological differentiation of species by means of chiasma studies, even though the species from which they arose may have shown significant differences in chiasma frequency. There is no evidence from these studies that chiasma frequency in the polyploid oats is reduced by competition in pairing.

*Chiasma Frequencies in Triploid, Pentaploid and Hexaploid Hybrids*

In the pure species every chromosome has its identical mate, but in the species hybrids a given chromosome may or may not have a homologous mate. The degree of homology may conceivably vary, and the closer the relationship between the parental forms, the greater the chance of homology between their chromosomes. But again the questions of environment, nature of the genes that affect pairing and chiasma formation, and, in addition, structural chromosome changes, are all complicating factors. A lack of pairing does not necessarily indicate a lack of homology, but close synapsis must indicate a considerable degree of homology between the chromosomes that pair. Chiasma count at metaphase is a less direct indication of homology than observations on synapsis, but it is the best criterion available for this material as it is not possible by present methods to study the whole chromosome complement critically at early stages.

In the triploid hybrid metaphase pairing was close. In all of the observed cells, there were at least seven bivalents, presumably seven chromosomes of *A. strigosa* paired with seven chromosomes of *A. barbata*. In half of the observed cells, however, more than 14, and usually 16, univalents were involved in chiasma formation. The average number of chiasma per bivalent in the hybrid, as calculated on the basis of 7.5 bivalents per cell, is 2.40. The average number in the parental strains is 2.19 and 2.16 respectively. The higher number in the hybrid is somewhat unexpected, but is perhaps due to different methods of fixation. It is at any rate evident that there exists a very considerable degree of homology between the chromosomes of *A. strigosa* and seven of the chromosomes of *A. barbata*. In addition to the pairing of seven chromosomes from each parent, one, two or three of the supernumerary chromosomes of the tetraploid parent entered at times into pairing relationships. Cells with eight bivalents were observed, and in the same hybrid Nishiyama (12) has found cells with nine bivalents. In 10 cells chosen at random, three trivalents were noted. Although no quadrivalents were observed in the material examined, Nishiyama (12) reports rare quadri-

valents in the hybrid he studied. He also found as many as three trivalents in one cell. Such behavior might be explained by the following scheme:




(i) Seven chromosomes of the diploid are homologous or very nearly homologous to seven tetraploid chromosomes.

(ii) Three supernumerary chromosomes of the tetraploid have no homologous or partially homologous mates among the diploid chromosomes.

(iii) Three supernumerary chromosomes are partially homologous to three chromosomes of the diploid. Hence the occurrence of as many as three trivalents in one cell.

(iv) Postulate duplications between two pairs of the supernumerary chromosomes, and autosynapsis as well as the appearance of the occasional quadrivalent configuration is accounted for.

Observations on the triploid hybrid lead to the conclusion, already suggested by comparative study of chiasma frequency of *A. barbata* and *A. strigosa*, that these two species are very closely related.

Pairing in the pentaploid hybrid as judged by chiasma formation, is looser than in the triploid hybrid discussed above. More than half the potential bivalents, that is 14 per cell, had no chiasmata, 44.5% had one, and 4% had two. The number of bivalents per cell varied from 5 to 11 (Fig. 10*a*). Nishiyama (12) in the pentaploid hybrid *A. barbata* × *A. fatua* found 2 to 11 bivalents, including 1 to 4 trivalents, and in *A. barbata* × *A. sterilis* 7 to 13 bivalents, including 0 to 4 trivalents. Emme's (5) results correspond with those of Nishiyama. In general then, crosses involving *A. barbata* or *A. abyssinica* and *A. sterilis* or *A. fatua* indicate either a lack of close relationship between the chromosomes of these tetraploid and hexaploid species, or the lack of genes favorable to synapsis or chiasma formation. The evidence is in favor of the former alternative, for our results with pure species suggest that a concentration of genes in polyploids is likely to result in the maximum chiasma formation between homologous chromosomes. It should be noted, however, that in this case the tetraploid parent was probably an aberrant form with chiasma formation lower than that normal to the species. Trivalents were seen in several of the pentaploid cells (Fig. 10*b*).

The hexaploid hybrid *A. sterilis* × *A. sativa* var. Radnorshire Sprig showed practically the same chiasma frequency as did its low frequency parent *A. sterilis*, but the $\chi^2$ test indicates that the hybrid and *A. sativa* var. Radnorshire Sprig do differ significantly in respect to chiasma formation. Although *A. sativa* and *A. sterilis* are not commonly considered very closely related species, the cytological evidence of a difference between them is slight. Close pairing in the hybrid may have been due in some small measure to autosynapsis between partially homologous chromosomes present through polyploidy, although multivalents and univalents were seen in only about 20% of the cells. However, this cannot be established cytologically, as the origin of the paired chromosomes cannot be determined. The cytological study of species reveals no significant difference, so that what little evidence is afforded by these studies is based upon the difference between the hybrid and its parent, *A. sativa* var. Radnorshire Sprig. It may be noted that certain characters of the variety Radnorshire Sprig suggest affinities with *A. byzantina* C. Koch, a species associated with *A. sterilis* in the relationship schemes of various authors.

Nishiyama (12) found in his hexaploid hybrids sometimes one to four univalents in one cell. In the hexaploid hybrid under consideration in this paper, univalent chromosomes were not observed in cells whose full chromosome complement could be distinguished with certainty. A few cells, damaged by pressure, showed two univalents.

Chiasma formation in the three different hybrids is represented graphically in Fig. 12.

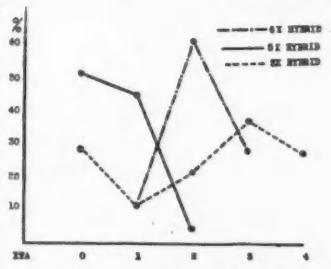


FIG. 12. *Percentage of bivalents with different numbers of chiasmata in the three hybrids studied.*

## Conclusions and Summary

It is possible that chiasma frequency at metaphase is influenced by external and internal environment, competition in pairing in polyploids, genes affecting synapsis and chiasma formation, structural hybridity and the degree of homology between members of a bivalent.

The only direct indication of relationship between the oats species studied is homology of chromosomes as judged by pairing behavior in the hybrid. A similarity of genes that affect synapsis and chiasma formation may indicate relationship between pure species. The cytological determination of relationship is complicated by the remaining factors, and by the fact that intraspecific gene differences may be as great as interspecific ones.

A comparison of different species of *Avena* with respect to chiasma formation reveals significant differences between certain diploid species. The closely allied forms *Avena strigosa* and *A. brevis* are alike, but both differ significantly from *A. Wiestii*. The differences between the tetraploid forms studied are of questionable significance, those between the hexaploid forms insignificant. It is suggested that a concentration of genes favoring synapsis and chiasma

formation is responsible for the lack of significant differences between polyploid forms which are not closely related. The relationships between diploid and tetraploid species are also indicated by similarities in chiasma frequency. *A. brevis*, *A. strigosa* and *A. barbata* show similar chiasma formation, while *A. Wiestii* resembles *A. abyssinica* in this respect.

The hybrid investigations yield results in accord with those of previous workers. In the triploid and hexaploid hybrids, pairing is close, in the pentaploid hybrid relatively loose.

Close pairing in the triploid is considered an indication of close relationship between *A. strigosa* and *A. barbata*, a conclusion also supported by chiasma studies in the pure species. Low chiasma formation in the pentaploid hybrid *A. abyssinica* × *A. sterilis* suggests lack of homology between parental chromosomes. Chiasma formation in the hexaploid hybrid is high. While it may be that this results in part from autosynapsis, the cytological evidence fails to indicate anything more than a very slight non-homology between chromosomes of the parental species, *A. sativa* var. Radnorshire Sprig and *A. sterilis*.

## Acknowledgments

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# STUDIES IN THE VARIABILITY OF TUBERCLE BACILLI.

## VIII. BCG.[1]

By G. B. Reed,[2] J. H. Orr[3] and Christine E. Rice[4]

## Abstract

Five cultures of BCG have been shown to consist primarily or entirely of R type tubercle bacilli and to resemble R types from other sources. When received one culture contained a few S types which were isolated. The other four cultures appeared to consist entirely of R types. After a long series of cultures, particularly in slightly alkaline Proskauer and Beck's fluid, dissociation occurred and S types were isolated from two strains; no stable S types could be recovered from two additional strains.

The R, or ordinary BCG produced only localized infections, the S forms produced fatal progressive tuberculosis in guinea pigs. The degree of tissue involvement in fatal cases was generally less than with typical virulent tubercle bacilli.

By means of complement fixation reactions it was shown that the S form recovered from BCG cultures, like typical S virulent mammalian tubercle bacilli, contains a specific antigenic substance which is lacking in the ordinary BCG and other R forms of the species. Animals immunized with S organisms are shown to develop a specific S antibody which is not present in the serum of animals treated with R organisms or the ordinary form of BCG. It is suggested that this lack of antigenic substance in the BCG militates against its effectiveness as an immunizing agent.

## 1. Introduction

The greater part of the now voluminous literature on the stability and avirulence of the bacillus of Calmette and Guérin is in agreement with the view of Calmette (8) and his associates that this strain of the tubercle bacillus is a stable, true-breeding, avirulent or nearly avirulent organism. There is almost the same unanimity of opinion that vaccination with living cultures of this organism imparts a degree of resistance against virulent tubercle bacilli. In the hands of a few observers, especially Petroff (17–21), Begbie (4), Watson (37, 38), Sasano and Medlar (32), Dreyer and Vollum (10), Seiffert (33), the culture has exhibited sufficient variability that virulent types have been isolated, or progressive disease produced in animals. In his more recent work Watson (38) has found that the virulence, rarely manifested in the earlier cultures, has completely disappeared in their descendants since 1928–29.

It has now been amply demonstrated that tubercle bacilli and related acid-fast species of bacteria may exhibit variation similar to that shown by other bacterial species. Petroff, Branch and Steenken (19, 20) and later Petroff and Steenken (21) described two colony forms exhibited by several cultures of human, bovine and avian tubercle bacilli. They found that virulence was generally associated with one colony type, the S, and low virulence generally associated with another type, the R. This was later confirmed by

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*Contribution from the Department of Bacteriology, Queen's University, Kingston, Ontario, Canada, with the co-operation of the Associate Committee on Tuberculosis, National Research Council, Ottawa, Canada.*

[2] *Professor of Bacteriology, Queen's University.*
*Assistant Professor of Bacteriology, Queen's University.*
*Research Fellow in Bacteriology, Queen's University.*

Begbie (4), in earlier papers in this series, by Reed and Rice (26, 27), Reed (24), by Kahn and Schwarzkopf (13, 14) and others. In the earlier papers from this laboratory it was shown that in a considerable series of cultures of tubercle and other acid-fast species, recently isolated virulent cultures, or cultures which had long maintained high virulence, grew in S colonies or exhibited a preponderance of S colonies, grew in a characteristic habit in fluids and exhibited acid agglutination only in strongly acid buffer solutions; avirulent cultures or cultures with a history of loss of virulence grew in R colonies on solid media, in a characteristic habit in fluid media and exhibited acid agglutination when suspended in relatively less acid buffer solutions. Cooper (9) found that S and R forms from the same strain differ in chemical composition; Kahn and Schwarzkopf (13) demonstrated differences in electrophoretic potentials and Reed and Gardiner (25) found differences in electrophoretic potential, acid agglutination and the isoelectric points of suspensions of S and R types. An apparently more significant difference between the R and S types was demonstrated in the writers' earlier papers, Rice (29), Rice and Reed (31), Reed, Rice and Gardiner (28); it was shown that the S types of tubercle bacilli contain an antigenic substance which is lacking in the R types.

In the original observations of Petroff and his associates (17–20) it was found that BCG cultures produced primarily R colonies. In certain of these cultures, however, they were able to demonstrate a small proportion of S colonies which were isolated and found to be similar in colony structure and in virulence to other S mammalian tubercle bacilli. This was confirmed by Begbie (4) and more recently by Seiffert (33). On the other hand several observers, including Birkhaug (2) who has reviewed the earlier observations, have isolated S-like colonies but found them to be as avirulent as the original BCG. Others contend that the S-like forms isolated by Petroff are human tubercle bacilli and, as BCG was developed from a bovine strain, regard the S forms in his cultures as contaminations. This appears to have been Calmette's (8) opinion, but it is not shared by those most familiar with Petroff's work.

Any differentiation of strains of tubercle bacilli on the basis of colony structure is at present very difficult owing to the fact that no two workers appear to be in agreement as to the structure of S and R colonies. Seiffert (33), who has probably made the most exhaustive study, describes several sub-groups of both S and R types recovered from BCG cultures. Our own results with BCG indicated in more detail below, are, in part, in agreement. What we regard as R types, Figs. 1 and 2, have been regularly recovered from BCG cultures. These colonies appear to be the same as Seiffert's (33) Hohe Haufchenform and the R forms described by Petroff (17–21) and others who have worked with this culture. In the course of long cultivation of cultures of these R types most of the R sub-types of Seiffert have been observed but we have not succeeded in stabilizing them to a sufficient degree to obtain true breeding cultures. As noted in a later section, one of Seiffert's R types in particular so closely resembles S types that at times there has been considerable confusion.

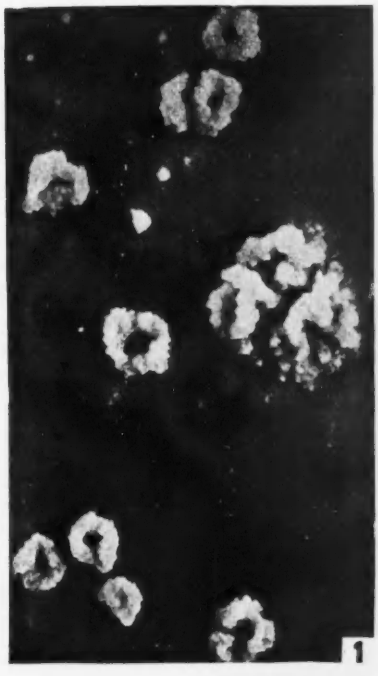
The S colonies which we have obtained from two of the five cultures of BCG studied, Figs. 4, 5, 6 and 7, as just noted, resemble S colonies obtained from other mammalian strains of tubercle bacilli (Reed and Rice (27)). They are apparently the same as Seiffert's (33), Trockene Halbkugelform or Trockene Schiebform and the S forms described by Petroff (17, 18) and Begbie (4) as recovered from BCG. Fig. 9 indicates one S colony from a bovine type culture, Vallée strain, and Fig. 10 one S colony from a human type culture, a strain recently isolated from sputum, and grown under the same conditions as the S forms recovered from the BCG cultures. As in the case of the R forms we have from time to time observed the other S forms described by Seiffert but have not succeeded in isolating them in stable form. Birkhaug (3) on the other hand describes an "S form" recovered from BCG as "hemispherical, of a creamy consistency and smooth surface." This does not appear to agree with any other description of S forms of mammalian tubercle bacilli except possibly Seiffert's (33) Feuchte Halbkugelform. It is not apparent whether this is the "S type" of BCG which Birkhaug described in an earlier paper (2) as avirulent for guinea pigs.

Several investigators, without including a study of dissociation from the point of colony structure, have reported a general increase in the virulence of BCG cultures (Watson (37, 38)). In a similar manner Sasano and Medlar (32) found that serial cultures in Sauton's fluid containing normal rabbit serum and Dreyer and Vollum (10) found that cultures in broth under semi-anaerobic conditions resulted in greatly enhanced virulence. Boquet (7) however was unable to confirm either of the last two results.

In the present study some five strains regarded as BCG have been examined. As noted in the following sections they were obtained from Dr. Petroff of Trudeau, Dr. Watson of Ottawa, Dr. Rankin of Alberta, Dr. Parizeau of Montreal and M. Nègre of Professor Calmette's laboratory at the Pasteur Institute. These cultures were received and have been examined over a period of some five years. They have not all been subject to the same treatment or examined for the same periods so that the results with the several cultures cannot be directly compared. The general procedure has been to start from the cultures as received, a series in fluid media, generally Proskauer and Beck's synthetic medium and to make a number of plate cultures on gentian-violet egg media and generally also on Petragnani's egg-potato-malachite-green medium. In most instances the fluid cultures have been carried by serial transfers through many cultural generations with side cultures to solid media from time to time to determine the colony form and with frequent guinea pig inoculations to test the virulence.

## 2. Results with the Trudeau Strain of BCG

Dr. Petroff was kind enough to send us a culture of BCG, presumably one of those used in his study of dissociation (17–21). The primary series of plates on gentian-violet egg media made from this culture developed a majority of R colonies. These were similar in structure to the R types in all five strains



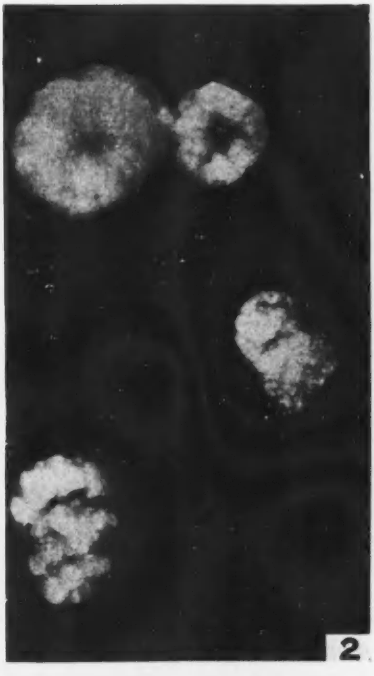
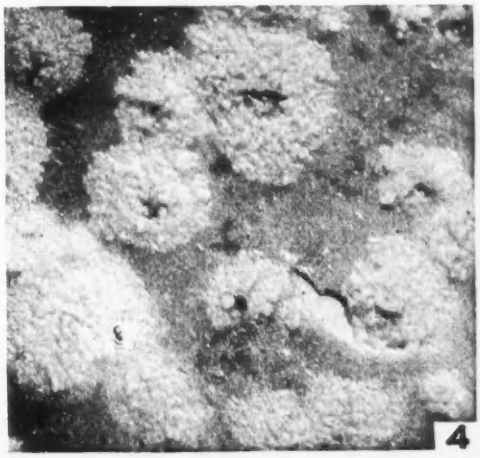
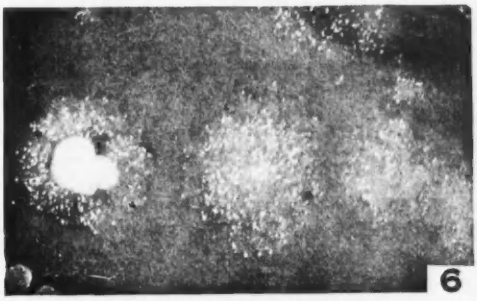
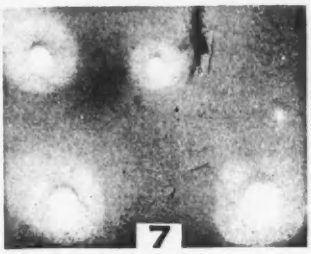



PLATE I. BCG COLONIES: FIGS. 1 AND 2. *Characteristic R colonies from the Ottawa and Alberta cultures.* FIG. 3. *Two cultures of the Ottawa strain on Proskauer and Beck's fluid media. At the left—a characteristic R BCG growth; at the right—an R-like mass of growth with a larger area of spreading veil from which an S type was eventually isolated.*





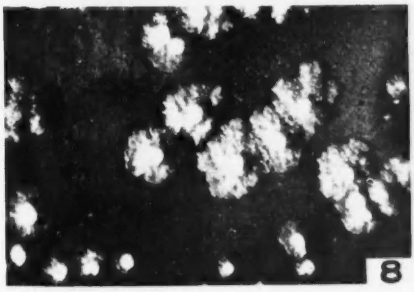



PLATE II. BCG COLONIES. FIG. 4. *S colonies isolated from the Trudeau culture.* FIG. 5. *R and an intermediate or S colony spreading out from an R colony, from the Alberta culture.* FIG. 6. *At the left—an R colony with peripheral zone of S growth; right—2 S colonies.* FIG. 7. *S colonies isolated from the Ottawa culture.* FIG. 8. *R colonies from the Montreal culture, resembling Seiffert's Feuchte Körnchenform, frequently confused, when young, with S.* FIG. 9. *S colony bovine tubercle bacilli, Vallée strain, grown under the same conditions as the BCG.* FIG. 10. *S colony human tubercle bacilli recently isolated from sputum of an active case of pulmonary disease and grown under the same conditions as the BCG.*

examined and appear to be identical with those figured by Petroff (17–21) and Begbie (4), and closely resemble R colonies previously described from other strains of tubercle bacilli (Reed and Rice (26, 27) ). (Figs. 1 and 2.) The primary plates, however, also developed a considerable number of very definite S colonies.

The most S-like were fished and replated; after one to six platings from single colony isolations, strains were obtained which produced only S colonies for several serial cultures on gentian-violet egg media. These S colonies (Fig. 4), with their much flattened conical structure, thin spreading and irregular outline and finely granular surface resemble S colonies from many other cultures of mammalian tubercle bacilli (Reed and Rice (27) ), and contrast sharply with the R-like colonies of the usual BCG culture (Figs. 1 and 2). In fluids such as Sauton's or Proskauer and Beck's media, the more or less stabilized S first grew as a thin spreading veil which later became thickened and folded in constrast to the ordinary BCG which grows as a thick granular pellicle readily separating into island-like masses. The S forms as shown in section 8 contained the S specific antigenic substance absent in the R type or ordinary BCG.

Doses of one to five milligrams of the S culture injected intracardially or intraperitoneally into guinea pigs produced generalized tuberculosis and death in three to six weeks.

It seems evident, therefore, that this culture when received consisted of two types, distinguishable by colony structure, antigenic content and virulence.

### 3. Results with the Ottawa Strain of BCG

Dr. Watson, of the Canadian Department of Agriculture very kindly provided us with a culture of BCG marked "80B", evidently one of those used in his study of BCG reactions of calves (37, 38).

In sharp contrast with the Trudeau BCG the initial series of cultures from this strain developed only the R type of colony (Figs. 1 and 2). It is possible that more plates might have demonstrated some variability but it could not be found among some 50,000 to 100,000 colonies compared in the original series and, as shown in more detail in Table I, several guinea pigs inoculated with five-milligram doses failed to develop generalized tuberculosis in three to six months.

Proskauer and Beck's fluid media made slightly alkaline, pH 7.4 to 8.0 in different lots, was inoculated from the original culture and carried through some 30 serial cultures over a period of three years, with numerous side cultures in this or other fluid media and to plates for colony structure studies. Animal inoculations were made direct from many of the Proskauer and Beck serial cultures or from the side series.

In the serial cultures in fluid media advantage was taken of the difference in the habit of growth of S and R forms. As described by Petroff (17, 18),

and discussed in some detail in an earlier paper by Reed and Rice (27) human and bovine tubercle bacilli of the R type grow on the surface of many fluid media as a thick granular pellicle readily separating into island-like masses during the first two to three weeks of growth. In conspicuous contrast, especially in very young cultures, the S type tends to grow in a continuous, flat, spreading, veil-like pellicle. Accordingly any tendency to veil-like growth was taken as suggestive of the appearance of an S variant. This tendency was generally most readily observed where rather large volumes of culture fluid were used (100 to 300 cc. amounts in 250 cc. or litre Erlenmeyer flasks). These veil-like areas appeared as marginal outgrowths from granular masses or as continuous connections between islands of granular pellicle (Fig. 3). In the serial transfers to Proskauer and Beck's fluid, as well as to the side cultures on plates, inoculum was always taken from the more definite veil-like growths.

The most important features of this series of cultures are summarized in Table I. It will be observed that the first 14 serial cultures on the Proskauer and Beck's medium resulted in the characteristic heavy, granular, island type of pellicle; all the plates developed R colonies characteristic of typical BCG. The small number of guinea pigs inoculated developed only the usual localized BCG type of reaction. It is quite possible that with large numbers of guinea pigs, as used by Uhlenhuth and Seiffert (35) or if the animals had been kept under observation for long periods as in certain of Watson's (37, 38) experiments, some generalized cases would have appeared. But within the limitations of the experimental procedure this culture behaved for these 14 cultural generations, approximately two years, as a characteristic BCG.

As noted in Table I, the cultures from the ninth serial transfer developed some traces of the thin spreading veil which was used for inoculum for the subsequent cultures. Although the initial plates from this veil failed to yield colonies different from the heavier pellicle, the fifteenth serial fluid culture when plated developed a few apparently S colonies. Successive platings from the veil resulted in increase in this type of growth at the expense of the heavy granular or island habit, and by the twenty-eighth or twenty-ninth culture plates, produced exclusively the S type of colonies. At the same time that a preponderance of the colonies developed in the S form, doses of from one to five milligrams injected intraperitoneally into guinea pigs resulted in generalized tuberculosis and death in three to eight weeks.

S colonies were also isolated from the earlier plates on which they appeared and, as in the case of the Trudeau culture, after several replatings from single S colony isolations S strains were established similar to those obtained by veil transfers in the serial fluid cultures (Figs. 6 and 7).

The S forms, obtained by either procedure, as in the former case, were distinguished from the R or ordinary BCG by acid agglutination, by antigenic structure (section 8) as well as by the differences in colony structure and virulence.

TABLE I

COLONY STRUCTURE AND VIRULENCE OF THE OTTAWA BCG DURING THE COURSE OF SERIAL CULTURES ON PROSKAUER AND BECK'S ALKALINE FLUID. VEIL WAS USED AS FAR AS POSSIBLE FOR INOCULUM

| No. of generations | Special cultures in P. and B. media | Colony structure, on solid media | Guinea pig reaction |
|---|---|---|---|
| 1 | Island type | All R | 6 pigs, 5 mg. intrap. No generalized Tb. after 3 mo. |
| 2 to 8 | Island type | All R | |
| 9 | Islands, trace veil | All R | 6 pigs, 5 mg. intrap. 2 died 6 and 20 days with extensive Tb. No generalized Tb. after 3 mo. in remaining 4. |
| 10 | Islands, trace veil | All R | |
| 11 | Islands, trace veil | All R | 6 pigs, 5 mg. intrap. No generalized Tb. after 3 mo. |
| 12 to 13 | Islands, trace veil | All R | |
| 14 | Islands, trace veil | All R | 6 pigs, 5 mg. intrap. No generalized Tb. after 1 to 6 mo. |
| 15 | Islands, trace veil | Mostly R, few S | |
| 16 | Islands, trace veil | | |
| 17 | Islands, trace veil | Mostly R, few S | |
| 18 | Islands, trace veil | Mostly R, few S | 6 pigs, 5 mg. intrap. No generalized Tb. after 1 to 5 mo. |
| 19 | Islands and veil | Mostly R, few S | |
| 20 | Islands and veil | R and S | |
| 21 | Islands and veil | Mostly S, few R | 4 pigs, 5 mg. intrap. 1 died intercurrent. No generalized Tb. in 2 at 50 and 98 days. Tb. of glands, spleen, liver, in 1 at 8 mo. |
| 22 | Islands and veil | Mostly S, few R | 2 pigs, 5 mg. intrap. Generalized Tb. at 80 and 74 days. |
| 23 | Islands and veil | Mostly S, few R | 4 pigs, 5 mg. intrap. 1 died intercurrent. 3 died generalized Tb. at 101, 30, 106 days. |
| 24 and 25 | Islands and veil | Mostly S, few R | |
| 26 | Veil, few islands | Mostly S, few R | 6 pigs, 5 mg. intrap. 1 died intercurrent. 5 died generalized Tb. at 60, 46, 21, 73, 63 days.<br>3 pigs and 2 rabbits inoc. from spleen of latter pig—intrap. 3 pigs died generalized Tb. at 6, 16, 18 days. 2 rabbits died generalized Tb. 18, 24 days. |
| 27 | Veil, very few islands | Mostly S, few R | |
| 28 | All veil | All S | |
| 29 | All veil | All S | |
| 30 | All veil | All S | 6 pigs, 1–3 mg intrap. 2 died intercurrent. 4 died generalized Tb. at 29, 20, 38, 52 days. |

It is perhaps surprising that this culture should have initially and for two years of serial transfers exhibited only R colonies and the usual BCG type of virulence, in view of the fact that Watson (37, 38) reported progressive tuberculosis in certain of his experimental animals inoculated with the culture. In our earlier studies on the dissociation of R type tubercle bacilli it was

repeatedly observed, Reed and Rice (27), Reed (24), that the S organisms first recovered from dissociating cultures were extremely unstable, and readily reverted to the R form. The same condition appears to be even more characteristic of the S forms recovered from this culture. In many instances cultures which behaved for one to several cultural generations as an S type again reverted to the R form. It is therefore conceivable that this particular culture when used by Watson for his animal inoculations contained S elements, but of an unstable character. In any case it is evident that the culture when received, and for more than a year of serial transfers, behaved like the many descriptions of BCG. It then gradually developed more and more S elements and increased in virulence for guinea pigs. This appears to be an ordinary instance of R to S bacterial variation as observed in many other species.

### 4. Results with the Alberta Strain of BCG

Dean Rankin, University of Alberta, was good enough to send us a culture of BCG, evidently the culture used in his extensive studies on the vaccination of calves (22, 23). As in the case of the Ottawa strain this culture, when received from Dr. Rankin, November, 1930, yielded on the primary plates only R types of colonies, and a small group of guinea pigs inoculated from the primary cultures developed only the typical localized BCG infection. As in the former case, this strain has been carried in serial cultures in Proskauer and Beck's slightly alkaline fluid. Where veil or any suggestion of veil-like pellicle appeared it was used for inoculum. Results from the more significant part of the series are summarized in Table II. As indicated there, in the initial culture on Proskauer and Beck's fluid, some traces of veil-like pellicle appeared, yet plates from the first five serial cultures failed to yield any definite S colonies, and guinea pigs injected with five-milligram doses developed only localized tuberculosis. The sixth to the tenth serial culture developed slightly more veil-like pellicle, and plate cultures showed more S-like colonies, yet five-milligram doses in guinea pigs failed to produce any rapidly developing or generalized tuberculosis. The eleventh serial culture did produce generalized infection in four guinea pigs, and plates from this culture developed a small proportion of characteristic S colonies. Several of these were fished and serial cultures carried on from single S colony isolations on gentian-violet egg media. As noted in Table II the third and fourth cultures of the latter series were largely or entirely S in colony structure (Fig. 5), and doses of five to one-tenth milligrams injected into guinea pigs resulted in rapidly progressive tuberculosis.

As in the former instances the establishment of stable S cultures was only effected after many serial transfers from single S colonies. Many S-like colonies when isolated failed to produce S progeny; many produced mixtures of types, but persistent selection yielded S cultures similar to those isolated from the other strains. As in other instances these S forms have been distinguished by antigenic structure (section 8) as well as by colony structure and virulence.

TABLE II

COLONY STRUCTURE AND VIRULENCE OF THE ALBERTA STRAIN OF BCG DURING THE COURSE OF SERIAL CULTURES IN PROSKAUER AND BECK'S FLUID, AND SINGLE COLONY ISOLATION SERIES ON SOLID MEDIA

| No. of generations | Serial cultures in P. and B. media | Colony structure | Guinea pig reaction |
|---|---|---|---|
| Serial cultures in Proskauer and Beck's:— | | | |
| 1 | Islands, trace veil | R only | 6 pigs, 5 mg. intrap. killed after 3 mo. No generalized Tb. |
| 2 | Islands, trace veil | R only | |
| 3 | Islands, trace veil | R only | |
| 4 | Islands, trace veil | R only | 6 pigs, 5 mg. intrap. 2 died intercurrent 38, 42 days. 4 killed 200 days no generalized Tb. |
| 5 | Islands, trace veil | R and few S-like | 2 pigs, 5 mg. intrap. 2 died interc. at 60 and 70 days, no generalized Tb. |
| 6 to 10 | Islands, trace veil | R and few S-like | |
| 11 | Islands and veil | R and S-like | 4 pigs, 5 mg. intrap. generalized Tb. at 90 days. |
| Serial cultures from single colony isolations beginning from the 11th Proskauer and Beck culture:— | | | |
| 12 | | R and S | |
| 13 | | Mostly S, few R | |
| 14 | | Mostly S, few R | 3 pigs, 5 mg. intrap. died with generalized Tb. at 28, 77, 83 days. 3 pigs inoculated from latter, died with generalized Tb. at 41, 66, 69 days. |
| 15 | | Mostly or entirely S | 2 pigs, 1 mg. intrap. died with generalized Tb. at 41, 60 days. 2 pigs 0.5 mg. intrap. died with generalized Tb. at 70 and 134 days. 2 pigs, 1 mg. intrap. died with generalized Tb. at 138, 175 days. |

It seems apparent then that the Alberta culture when received behaved like a characteristic BCG or R form and that the changes just recorded represent another instance of R to S variation.

### 5. Montreal Strain of BCG

In December, 1931, Dr. Parizeau, Vice Dean, University of Montreal, was kind enough to send us a culture of BCG which he had received from the Pasteur Institute in 1928 and "reproduced every three weeks according to Professor Calmette's technique."

A large series of plates of gentian-violet egg media inoculated as soon as the culture was received, exhibited only R type colonies. Cultures were then started in Proskauer and Beck's fluid and carried through 27 serial transfers with the results indicated in Table III.

TABLE III

COLONY STRUCTURE AND VIRULENCE OF THE MONTREAL STRAIN OF BCG DURING THE COURSE OF SERIAL CULTURES ON PROSKAUER AND BECK'S FLUID MEDIA

| No. of generations | Serial cultures in P. and B. media | Colony structure | Guinea pig reaction |
|---|---|---|---|
| 1 | Islands, trace veil | | |
| 2 | Islands, trace veil | R, very few S? | 2 pigs, 5 mg. intrap. Generalized Tb. yielding cultures—R and S. 2 pigs, intrap. from S? colonies of first, extensive Tb. yielding cultures—R and S. 4 pigs, intrap. from S? colonies of second—no Tb. after 3 mo. |
| 3 | Islands, trace veil | R, very few S | 2 pigs, 5 mg. intrap. No Tb. after 3 mo. |
| 4 to 6 | Islands, trace veil | | |
| 7 | Islands, trace veil | R, very few S | 3 pigs, 5 mg. intrap. No reaction after 3 mo. |
| 8 to 12 | Islands, trace veil | | |
| 13 | Islands, trace veil | R, very few S | 4 pigs, 5 mg. intrap. Extensive Tb. yielding cultures—R and S? 2 pigs from S colonies of first—no Tb. after 11 mo. |
| 14 to 26 | Islands, trace veil | | |
| 27 | Islands, trace veil | R and very few S? | 6 pigs, 5 mg. intrap. Localized Tb. only after 3 mo. |

From the first generation in Proskauer and Beck's medium this culture produced a small proportion of veil-like growth in contrast to the more massive R or island type of pellicle but, as indicated in Table III, notwithstanding 26 serial transfers from the veil-like structure, over a period of two years, the growth habit remained essentially unchanged. This is a contrast to the two previously described strains.

Subcultures from the veil-like growth to solid media always gave a preponderance of R type colonies and a very small proportion of S-like colonies. In many instances the S-like colonies were cultivated serially from single colony transfers for many generations. Much confusion was experienced from the development of colonies which at first appeared characteristically S-like but on further growth developed thicker margins and a much more irregular surface (Fig. 8), to resemble Seiffert's (33) "Flache Körnchenform" —a type which is avirulent and definitely not S. In no instance was a true breeding and characteristic S type obtained.

The veil-like growth obtained from the second generation on Proskauer and Beck's fluid, injected intraperitoneally in five-milligram doses into two guinea pigs, caused generalized tuberculosis with extensive lesions in the omentum, spleen and liver, and death in 30 and 87 days. Cultures from the tuberculous lesions in these animals exhibited a majority of definite R colonies and a small proportion of S-like colonies. Two guinea pigs injected with five-mg. doses of these S-like growths developed extensive tuberculosis. Cultures

from the lesions in these animals also exhibited a majority of R colonies with a few apparently S forms though they were less characteristic. Four guinea pigs injected intraperitoneally with five-milligram doses of these atypical forms exhibited only localized tuberculosis in the omentum. Essentially the same reaction was produced by inoculating guinea pigs with veil from the thirteenth serial transfer, while guinea pigs inoculated from the twenty-seventh serial transfer in Proskauer and Beck's fluid developed only characteristic localized BCG reactions.

It seems apparent therefore that although this culture exhibited some tendency to variation and did gain enough virulence to produce generalized or extensive tuberculosis in two lots of guinea pigs, the period of study was too short, or the methods followed were not successful in separating a stable true-breeding S form.

### 6. Paris Strain of BCG

In November, 1929, Dr. Nègre of the Pasteur Institute was kind enough to provide us with a culture of BCG. A series of plates on solid media made from the primary culture developed only R colonies. The first eight serial cultures in Proskauer and Beck's fluid, and in the same medium containing 10% normal rabbit's blood, showed no tendency to vary from the typical, granular, island type of pellicle. Several small series of guinea pigs inoculated with five-milligram doses developed only localized reactions. The eighth to the fifteenth serial cultures exhibited traces of veil-like growth and plates from this veil developed a very small proportion of S-like colonies. In every instance, however, one to three subcultures of the S-like colonies resulted in reversion to typical R forms.

A longer series of observations would possibly have revealed more definite variation but it appears evident that this culture, like the Montreal strain, consists of more definitely fixed R types than the first three strains of BCG examined.

### 7. Pathogenicity of S Forms Recovered from BCG

As noted in the previous sections, definite S forms recovered from BCG cultures have produced generalized tuberculosis and death in guinea pigs and rabbits. All colonies with S-like form have not proved to be virulent. This has been especially true of the first S colonies isolated from cultures previously all R, and as already noted and listed in Tables I to III, many such supposed S colonies when subcultured have yielded only R progeny. The structure of the colony can only be taken as one feature of variation with which altered virulence may or may not be associated. The observed results might, on the other hand, be interpreted as evidence that slight variation in the organisms, too transitory to persist in subcultures or in inoculated animals, are registered in colony structure. At the same time established S colony types breed true to type in serial cultures and produce progressive disease in animals on serial passage.

With such established S types from the BCG cultures, intraperitoneal doses in guinea pigs of 0.1 to 5 mg. have resulted in progressive disease and death. But this progressive and fatal tuberculosis differs more or less conspicuously from the animal response to the usual virulent bovine and human strains of tubercle bacilli, in that massive involvement of many tissues does not occur. Following intraperitoneal inoculation of guinea pigs, the lesions have been most evident in the omentum, spleen, epididymis and liver, in the order named. Intracardial inoculations have always developed more widespread infections involving particularly the lungs, but the involvement of tissue has generally been distinctly less than is ordinarily the case with fatal generalized tuberculosis.

Guinea pig No. 587, for example, was given intraperitoneally two milligrams of a supposed S type recovered from the Ottawa culture of BCG. The animal died 63 days later. Post-mortem examination showed generalized thickening of the omentum with many nodular swellings which, on section, discharged creamy caseous material containing very large numbers of acid-fast bacteria presenting all the characteristics of tubercle bacilli. The spleen showed considerable enlargement and several gray to yellow nodules which, as in the case of the omental swellings, proved on section to contain caseous material and acid-fast bacilli. The liver presented a similar appearance though the nodules were much more scattered but here again acid-fast organisms appeared in smears from the nodules. The epididymis appeared reddish-brown in color and necrotic with scattered, minute nodules containing acid-fast bacteria. Many lymphatic glands showed slight enlargement. Other organs appeared from gross inspection to be normal.

Two guinea pigs were inoculated intraperitoneally and a rabbit intravenously with macerated nodules from the spleen of the above-mentioned guinea pig No. 587. The first guinea pig died 16 days and the second 30 days after inoculation, with lesions similar to those from which the inoculum was taken. The rabbit died 24 days after the injection and on post-mortem showed a miliary tuberculosis of the lungs, enlargement of the lymphatic glands, general thickening and nodular swelling of the omentum, and a few yellow nodules in the liver. Smears from the lungs and the nodular swellings in other organs exhibited moderately large numbers of acid-fast bacilli.

Many guinea pigs infected with 0.1- to 5- mg. doses of S forms isolated from the several BCG cultures have reacted in this manner. Some have died from the infection in as short a period as 12 days, some have survived for several months. Frequently more extensive involvement than that just described, has been encountered; other animals have died with gross lesions evident only in the omentum.

Characteristic S cultures were recovered from the lesions in many of these animals and progressive disease was produced on injection of the exudate or the tubercular tissue into other guinea pigs. In a few instances, notwithstanding the presence of large numbers of bacilli in the tissues and exudates

of animals dead of fatal progressive disease, it has been impossible to recover cultures or to produce infections with injected exudate. Such failures have been the exception.

It seems evident, therefore, that stable S forms recovered from BCG cultures are actively pathogenic. In the next section it is also shown that they possess a different antigenic structure from the R forms.

## 8. Antigenic Activity of BCG Cultures

In previous papers in this series it has been shown that S and R types of tubercle bacilli and other acid-fast species differ in antigenic structure as measured by complement-fixation (Rice (29), Rice and Reed (31), Reed, Rice and Gardiner (28), Rice, Orr and Reed (30).

The same technique as described in the former papers has been employed with BCG material. In complement-fixation, the total amount of complement reacting with the sensitized antigen and red cells to yield a final degree of haemolysis of 50%, has been used as the unit of comparison. The "specific fixation" with a particular antigen was obtained by subtracting from the amount of complement fixed by that antigen and an immune serum, the amount fixed by the same antigen in the presence of normal serum collected from the same animal previous to immunization. Although neither Shibata (34), Arena (1) or Boquet and Nègre (7) were able, in the presence of an excess of complement (two or more units), to demonstrate appreciable differences in the antigenic activity of extracts of BCG cultures and of preparations made from virulent strains, it was found possible by means of this more strictly quantitative technique to show that undissociated BCG cultures behave antigenically like other typical R cultures of tubercle bacilli, whereas dissociated S cultures, like other S cultures of tubercle bacilli, behave very differently from the R cultures.

Normal rabbits whose sera were low in nonspecific and anti-complementary properties, were given a series of inoculations with killed cultures of the various strains of BCG: two lots of animals with the characteristic BCG–R, Paris and Montreal strains; three lots of animals with the dissociated S forms of the Trudeau, Ottawa, and Alberta cultures; and one lot with a culture of the Alberta strain containing mostly R with a few S types.

These sera were tested in the presence of graded amounts of complement with a standard S antigen, an aqueous extract prepared from a typical S culture of tubercle bacilli and a standard R antigen, a similar extract made from a pure R culture of bovine tubercle bacilli. Both these antigens were used in previous work, Rice and Reed (31). The results of such a series of tests are assembled in Table IV. It is evident that the anti-R sera, the sera of rabbits immunized with the typical, or R, BCG of the Paris and Montreal strains, react to approximately the same degree with the S and the R antigens. Sera of three lots of rabbits immunized with dissociated S types from the Trudeau, Alberta, and Ottawa strains of BCG, the anti-S sera, react very

differently with the S and R antigens. With these anti-S sera, as shown in Table IV, the R antigen fixed from 45 to 60% of the amount fixed by the S antigen.

TABLE IV

A COMPARISON OF THE REACTIVITY OF TYPICAL S AND R MAMMALIAN TUBERCLE BACILLUS ANTIGENS WITH THE SERUM OF RABBITS IMMUNIZED WITH ORDINARY BCG–R ORGANISMS AND WITH DISSOCIATED S ORGANISMS AS MEASURED IN CUBIC CENTIMETRES OF COMPLEMENT FIXED SPECIFICALLY AT FIFTY PER CENT HAEMOLYSIS

| Antiserum | Antigen | Specific fixation | S/R Index | Antiserum | Antigen | Specific fixation | S/R Index |
|---|---|---|---|---|---|---|---|
| | | cc. | | | | cc. | |
| BCG–R, Montreal (1) | S | .0041 | 1.17 | BCG–S, Trudeau | S | .0064 | 2.13 |
| | R | .0035 | | | R | .0030 | |
| BCG–R, Montreal (2) | S | .0080 | 1.08 | BCG–S, Alberta (1) | S | .0032 | 2.28 |
| | R | .0074 | | | R | .0014 | |
| BCG–R, Montreal (3) | S | .0147 | 1.06 | BCG–S, Alberta (2) | S | .0143 | 1.64 |
| | R | .0139 | | | R | .0087 | |
| BCG–R, Montreal (4) | S | .0110 | 1.09 | BCG–S, Alberta (3) | S | .0143 | 2.17 |
| | R | .0101 | | | R | .0066 | |
| BCG–R, Montreal (5) | S | .0073 | 1.06 | BCG–S, Ottawa (1) | S | .0057 | 2.04 |
| | R | .0069 | | | R | .0028 | |
| BCG–R, Paris | S | .0090 | 1.13 | BCG–S, Alberta (4) | S | .0150 | 1.72 |
| | R | .0080 | | | R | .0087 | |
| BCG–S and R Alberta (5) | S | .0100 | 1.22 | | | | |
| | R | .0082 | | | | | |
| Average | S | .0091 | 1.10 | Average | S | .0098 | 1.88 |
| | R | .0083 | | | R | .0052 | |

In the earlier papers just quoted (29, 31), it was shown that in the sera of animals subjected to a long period of immunization with S tubercle bacilli, R antigens fix approximately half the antibodies fixed by the S antigen, whereas with the serum of animals similarly immunized with R organisms an equal amount of fixation occurs with R and S antigen. It was considered from the experimental data that the S organisms contain an S-specific and a species antigenic substance and that S antiserum contains a corresponding S antibody and a species antibody; while in contrast, the R organisms lack the S-specific antigen but possess the species antigen and the R antiserum contains only the species antibodies. Precisely the same arguments may be applied to the BCG data presented in Table IV. The cultures consisting of R types of BCG produced in rabbits only species antibodies which were fixed to an equal degree by known S and R tubercle bacillus antigens. The BCG cultures which had dissociated to S types, produced in rabbits both S-specific and species antibodies, as shown by the fact that the S antigen produced approximately twice as much fixation as the R antigen.

These data offer then, further confirmation of the R character of the original and typical strains of BCG. They also indicate that in the dissociation of the Trudeau, Alberta, and Ottawa strains, types have been produced which possess S-specific substances. Dissociation, as in other tubercle bacilli, has evidently been accompanied by a change in antigenic structure.

It is apparent from these experiments that the BCG as ordinarily used for immunization is, like other R types of tubercle bacilli, lacking in certain antigenic complexes present in virulent human or bovine cultures or in dissociated S forms of the BCG. To what extent the complement-fixing activity of tuberculous serum indicates resistance to the disease is unknown; the majority of workers do not consider such antibodies as essential elements in the defence against tuberculous infection. If any such connection does exist, however, the usefulness of an R vaccine such as BCG in protecting against virulent S organisms might be questioned, since no antibodies are developed against the S-specific substances. Even if complement fixing substances are not important in determining the degree of immunity, it is not improbable that the difference in antigenic structure may affect their general immune reactions.

## Discussion

These results apparently indicate that BCG cultures consist of R forms of tubercle bacilli and, in agreement with earlier observations on other well established R cultures of tubercle bacilli, they have proved to be highly stable. Had observations not been continued for one to three years we would have been in agreement with the majority of students of these cultures that the R form is a fixed type, judging by all except one of the cultures of BCG under examination. Continued observation, however, indicates that under possibly exceptional circumstances, R to S dissociation may occur. It seems highly unlikely that this change will ever take place under the usual conditions of the cultivation of BCG for vaccine purposes. From this point of view it might be considered that the observation of dissociation gives no indication of danger in the use of BCG vaccine. It does however call attention to the necessity of the most rigid control in the preparation of the vaccine and to the possibility of dissociation taking place in the animal body. No new evidence or discussion of the latter point has been introduced in this paper.

Probably the indication that the ordinary BCG culture, the R form, is antigenically different from the virulent or S tubercle bacillus offers the more serious commentary on the BCG vaccine problem. The R variants of other bacterial species, the pneumococci, the colon-typhoid group, have been shown to produce little protective effect in inoculated animals (12). It would not be surprising therefore to encounter a similar condition in tubercle bacilli. More definitely it has been shown in this paper that the BCG, in common with other R forms of the species, is lacking in certain antigenic complexes, demonstrable by complement-fixation reactions, which are present in virulent or S forms and that the serum of animals treated with killed S organisms contains a specific S antibody. Animals treated with R organisms lack this specific antibody. It is impossible to draw conclusions concerning immunity from complement-fixing reactions but, as previously discussed, it seems reasonable to anticipate that the lack of a specific S antigenic substance in the R organisms may seriously affect this general immunizing reaction.

## Conclusions

Five cultures of BCG have been shown to consist primarily or entirely of R type tubercle bacilli and to resemble R types from other sources.

One culture contained, when received, a few S types which were isolated. The other four cultures appeared to consist entirely of R types. After a long series of cultures particularly in slightly alkaline Proskauer and Beck's fluid, dissociation occurred and S types were isolated from two strains, no stable S types could be recovered from two additional strains.

The R or ordinary BCG produced only localized infections, the S forms produced fatal progressive tuberculosis in guinea pigs. The degree of tissue involvement in fatal cases was generally less than with typical virulent tubercle bacilli.

By means of complement fixation reactions it was shown that the S form, recovered from BCG cultures, like typical S virulent mammalian tubercle bacilli, contains a specific antigenic substance which is lacking in the ordinary BCG and other R forms of the species. Animals immunized with S organisms are shown to develop a specific S antibody which is not present in the serum of animals treated with R organisms or the ordinary form of BCG. It is suggested that this lack of antigenic substance in the BCG militates against its effectiveness as an immunizing agent.

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# THE IODIMETRIC ESTIMATION OF ACETONE[1]

By W. H. Hatcher[2] and J. F. Horwood[3]


## Abstract

The iodimetric estimation of acetone is directly related to two factors: (1) the enolization of acetone by alkali, and (2) the relative velocities of (1) and the reaction between alkali and iodine. The experiments necessary to emphasize these factors indicate the great rapidity of these two reactions at 25° C.


## Introduction

The use of iodine and alkali in quantitative estimations has been the subject of much investigation. The present communication is a continuation of a previous study by one of us (3) and represents a co-ordinated system of experiments designed to explain certain apparent inconsistencies previously noted. These inconsistencies may be summarized as follows:—

(1) Sodium hydroxide, acetone and iodine in potassium iodide solution give iodoform, etc., the completeness of the reaction being dependent upon the excess of alkali, the order of addition of reagents being that given;

(2) In the order acetone, iodine, sodium hydroxide, completeness is abnormally delayed;

(3) In the order iodine, sodium hydroxide, acetone, completeness of estimation is never achieved.

To avoid a large bibliography, only those publications having a distinct bearing on the problem will be mentioned.

## Procedure

Mixtures of acetone, sodium hydroxide and iodine were allowed to react in aqueous solution for a certain time, made just acid with dilute sulphuric acid, and immediately titrated with sodium thiosulphate. The conditions controlled were temperature, concentration, order of addition, and time of reaction.

## Reagents

The sodium hydroxide, iodine, iodate-free potassium iodide, and sodium thiosulphate were of the highest grade of purity obtainable.

The acetone used was correct in boiling point and contained no trace of aldehyde or acid. A weighed quantity was dissolved in distilled water and aliquots measured therefrom. The burettes and pipettes used were carefully calibrated, blank experiments performed, and all precautions taken to ensure accuracy. The highest error in titration was four-tenths of one per cent; usually it was about one-quarter of that.

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*Contribution from the Department of Chemistry, McGill University, Montreal, Canada.*

[2] *Associate Professor, Department of Chemistry.*

[3] *Demonstrator, Department of Chemistry.*

## Results

For the sake of brevity the experiments are summarized in Table I; here are given the experiment numbers, the concentration of reagents, the temperature, and the total volume of solution. These concentrations are expressed in gram molecules per litre for acetone and sodium hydroxide, and in gram

TABLE I

| Expt. No. | Concentrations | | | Temp., ° C. | Total volume, cc. |
|---|---|---|---|---|---|
| | Acetone | NaOH | Iodine | | |
| 1 | 0.0024 | 0.024 | 0.0234 | 25 | 200 |
| 2 | 0.0008 | 0.008 | 0.0078 | 25 | 600 |
| 3 | 0.0004 | 0.004 | 0.0039 | 25 | 1200 |
| 4 | 0.0008 | 0.012 | 0.0078 | 25 | 600 |
| 5 | 0.0008 | 0.016 | 0.0078 | 25 | 600 |
| 6 | 0.0008 | 0.024 | 0.0078 | 25 | 600 |
| 7 | 0.0008 | 0.048 | 0.0078 | 25 | 600 |
| 8 | 0.0004 | 0.024 | 0.0078 | 25 | 600 |
| 9 | 0.0004 | 0.024 | 0.016 | 25 | 600 |
| 10 | 0.0008 | 0.048 | 0.016 | 25 | 600 |
| 11 | 0.0008 | 0.048 | 0.0078 | 0 | 600 |

equivalents per litre for iodine. In each case the order of addition is acetone, sodium hydroxide, iodine.

The results at each time interval are not given but are found in Fig. 1, where the curves are numbered to correspond to the experiment numbers.

The experiments shown in Table I are correlated below together with the corresponding curves of Fig. 1, in order to show the influence of each factor:

I. Effect of dilution: Expts. 1, 2 and 3.

II. Effect of alkali concentration: Expts. 2, 4, 5, 6 and 7.

III. Effect of acetone concentration: Expts. 6 and 8.

IV. Effect of iodine concentration: Expts. 7 and 10, 8 and 9.

V. Effect of temperature: Expts. 7 and 11.

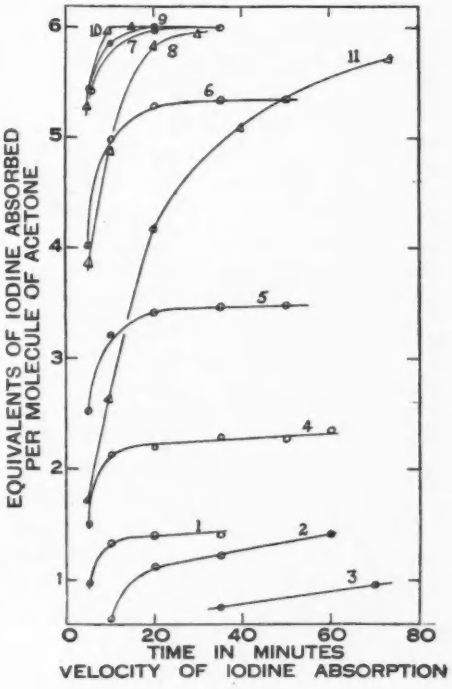


FIG. 1

In Fig. 1 the numbering of the curves corresponds to that of the experiments. Here time is plotted against gram equivalents of iodine used per gram molecule of acetone employed. Additional points left out of the curves are as follows: Curve 3, 1.42 equivalents in 315 min.; and curve 11, 5.90 equivalents in 130 min.

Mixtures having the concentrations of experiment No. 6 in Table I were made up in varying order of addition of reagents; the results are summarized in Table II.

TABLE II

| — | Order of addition | Iodine absorbed, % of the theoretical | Time |
|---|---|---|---|
| (A) | Acetone, Alkali, Iodine | 88 | 20 min. |
| (B) | Acetone, Iodine, Alkali | 59.8 | 16 hr. |
| (C) | Alkali, Iodine, Acetone | 54.6 | 41 hr. |

NOTE: *Temperature, 25° C.*

## Discussion of Results

A study of the curves in Fig. 1 reveals the following points: (i) a rapid reaction under favorable conditions; (ii) the apparent cessation of iodine absorption when the alkali concentration is less than 0.048 *N*. If the apparently final equivalents in Experiments 2, 4, 5, 6 and 7 be plotted against initial concentration of alkali, a straight line will be obtained which will indicate the final six equivalents of iodine when the alkali is about 0.03 *N*,—*i.e.*, about 35 mols of alkali for each one of acetone.

Table II shows the importance of the order of addition; this has further significance when the technique of addition is considered. In each case the first two reagents were added and the volume of solution was complete except for the addition of the third; the time of addition of all the reagents was about three minutes. In order (A), the acetone and alkali could stand together for as much as an hour without interfering with the final result; with order (B) the same is approximately true; whereas with order (C), delay in adding the acetone is fatal to the production of iodoform.

The reaction occurring here has been summarized in the well known equation:

$$4NaOH + 3I_2 + CH_3COCH_3 \rightarrow CHI_3 + 3NaI + CH_3COONa + 3H_2O$$

and is the basis of the Messinger method of acetone estimation (4). Since in neutral or acid solution acetone undergoes no rapid reaction such as occurs here, the effect of alkali on iodine solutions is obviously an important factor. An equally important factor is the action of alkali on acetone. Summarizing these we have the following reactions:

$$2K^+ + 2I^- + I_2 \rightleftarrows 2K^+ + I^- + I_3^- \qquad \text{rapid} \quad (1)$$

$$\left.\begin{aligned} &I_2 + OH^- \rightarrow HIO + I^- \\ \text{or}\quad &I_3^- + OH^- \rightarrow HIO^- + 2I^- \end{aligned}\right\} \qquad (2)$$

or the HIO may exist as $H^+$ and $IO^-$. This, according to Skrabal, is very rapid (5).

The much slower reaction

$$3I_2 + 6OH^- \rightarrow IO_3^- + 5I^- + 3H_2O \qquad (3)$$

enters subsequently.

According to Evans and Nicoll (1)

$$CH_3.CO.CH_3 \rightleftarrows CH_2.COH:CH_2 \qquad (4)$$

and this iso-acetone may be increased to 26.5% by alkali concentration of 0.0369 *N*, further alkali not affecting this equilibrium.

A picture may therefore be presented explaining the orders of addition and the effects of alkali, as follows:

*Order* (A). Acetone becomes enolized in direct proportion to the alkali concentration, the minimum concentration of about 0.03 *N* found previously agreeing well enough with that of Evans and Nicoll. Inasmuch as with 0.048 *N* alkali the reaction was almost complete in 10 min. (Fig. 1, curve 10), this acetone-iso-acetone equilibrium must be rapidly adjustable. Concurrently with this enolization, the rapid formation of HIO (perhaps, rather than NaIO or $IO^-$) completes the reaction by iodination, oxidation and fission of the chain at the ethylenic bond.

*Order* (B). Here the two main reactions (numbers 2 and 4) are in conflict, the rapid formation of hypoiodite partially removing the hydroxyl ion so essential for enolization, since the NaOH concentration was initially 0.024 *N*.

*Order* (C). The considerations above apply here more fully, as reaction No. 2 is well under way before the acetone is added,—even within the three minutes of addition.

That the reaction between sodium hydroxide and iodine dissolved in potassium iodide is rapid has been shown by mixing the two rapidly and taking their conductivity immediately: well within three minutes the conductivity had dropped to a value which it later maintained for a considerable period (2).

Apart, therefore, from offering an explanation of the iodimetry of acetone, these experiments give an idea of the rapidity of (i) enolization of acetone by alkali and (ii) the reaction between alkali and iodine.

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# CERTAIN REACTIONS OF GAMMA KETONIC ACIDS[1]

By C. F. H. Allen[2], J. B. Normington[3] and C. V. Wilson[4]


## Abstract

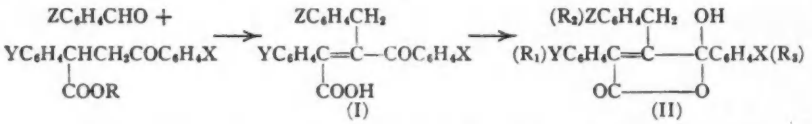
A considerable number of highly substituted acrylic acids or their lactols have been synthesized, and the Grignard reagent used to differentiate between the two possible structures. Acetyl chloride was found to be a satisfactory confirmatory agent, giving chlorides with the lactols, but not reacting with the open-chain acids. From the available evidence it is concluded that the differences may be attributed to *cis-trans* isomerism.

Two other series of ketonic acids were investigated with both reagents; the Grignard reagent indicated mostly open-chain structures. The use of acetyl chloride led to a variety of products; by varying the procedure, dimers of undetermined structure, unsaturated lactones, enolic acetates, and methyl esters were obtained.

Cyclohexanone gave cyclohexenyl acetate with acetyl chloride.

The mechanism of the reactions is discussed, as well as the evidence for the possible structures of derivatives of levulinic acid. A mechanism is suggested to account for the formation of enolic esters and unsaturated lactones of enolized ketonic acids.


When aromatic aldehydes are condensed by means of alkaline reagents with esters of $\alpha$-aryl-$\beta$-aroyl-propionic acids, the resulting products have been found to be acrylic acids or their lactols (3, 4).



It was noted (4) that when X = H, the acid only was produced, and that the latter showed no indications of a tautomeric cyclic structure. In order to learn whether there was any connection between the location and nature of substituent groups (X, Y, Z) and formation of open-chain or ring compounds, a considerable variety of compounds was prepared. The Grignard reagent was used to distinguish between the two types of structure. It was used in an apparatus designed to measure quantitatively both the amount of active hydrogen and addition (19, 20). In the earlier work it was found that the acids showed addition of one mole of reagent for each mole of gas evolved, whereas the cyclic substances added two moles per active hydrogen. A known unsaturated lactone of the same type showed two additions, indicating that the method was reliable with such substances. The results are recorded in Table I.

In two instances it was impossible to obtain check results. It will be noted that the mesityl homologue has the open-chain structure (one active hydrogen, one addition). Since 1,2-addition to mesitylenic ketones is un-

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*Contribution from the Department of Chemistry, McGill University, Montreal, Canada. (This paper was read at the meeting of the Royal Society of Canada at Quebec, May, 1934.)*

[2] *Assistant Professor of Chemistry, McGill University.*
[3] *Graduate Student, McGill University.*
[4] *Formerly Demonstrator in Chemistry, McGill University; now with the LaSalle Coke Company.*

TABLE I

RESULTS OBTAINED WITH THE LACTOLS OR KETONIC ACIDS IN THE GRIGNARD MACHINE

| $R_1$ | $R_2$ | $R_3$ | Active H | Addition |
|---|---|---|---|---|
| Acrylic acid series | | | | |
| $C_6H_5$ | $C_6H_5$ | $C_6H_5$ | 0.9 | 1.0 |
| $C_6H_5$ | $C_6H_5$ | $C_6H_4Cl$–4 | 1.1 | 2.1 |
| $C_6H_5$ | $C_6H_5$ | $C_6H_4Br$–4 | 1.0 | 1.9 |
| Piperonyl | $C_6H_5$ | $C_6H_4Cl$–4 | 1.1 | 2.1 |
| $C_6H_5$ | $C_6H_4Cl$–2 | $C_6H_5$ | 1.1 | 1.1 |
| $C_6H_5$ | $C_6H_5$ | $C_6H_4C_6H_5$–4 | 1.1 | 1.0 |
| Anisyl | $C_6H_5$ | $C_6H_5$ | 1.1 | 1.3 |
| Anisyl | α-Furyl | $C_6H_5$ | 1.1 | 1.1 |
| $C_6H_5$ | Piperonyl | $C_6H_4Cl$–4 | 1.3 | 1.1 |
| Anisyl | $C_6H_4Cl$–2 | $C_6H_5$ | 1.6, 1.3, 1.4 | 1, 4, 1.8, 1.9 |
| Anisyl | $C_6H_4OCH_3$–2 | $C_6H_5$ | 2.2, 1.9, 1.1, 1.6 | 0.3, 0.9, 1.6, 1.5 |
| $C_6H_5$ | $C_6H_5$ | Mesityl | 1.1, 1.1 | 1.1, 1.0 |
| $C_6H_5$ | $C_6H_5$ | $C_6H_4CH_3$–4 | 0.9 | 1.9 |
| $C_6H_5$ | $C_6H_4Cl$–2 | $C_6H_4Cl$–4 | 1.1 | 2.0 |
| Aryl aroyl propionic acids | | | | |
| $C_6H_5$ | $C_6H_5$ | | 0.9 | 1.0 |
| $C_6H_5$ | $C_6H_4F$–4 | | 1.1 | 1.1 |
| $C_6H_5$ | $C_6H_4Cl$–4 | | 0.9, 0.9 | 1.6, 1.5 |
| $C_6H_5$ | $C_6H_4Br$–4 | | 1.0 | 1.5 |
| $C_6H_5$ | $C_6H_4CH_3$–4 | | 1.0 | 1.9 |
| $C_6H_5$ | Mesityl | | 1.0 | 1.1 |
| Piperonyl | $C_6H_4Cl$–4 | | 1.1 | 2.0 |
| Benzal (7) | $C_6H_5$ | | 1.1 | 1.2 |
| α, γ-Diphenylcrotolactone (5) | | | 0.1 | 2.0 |
| α-Phenyl-β-benzyl-β-(4-chlorobenzoyl) propionic acid | | | 1.1 | 1.0 |

known, in this instance, by analogy with the behavior of a similar unsaturated ketone (18), the reagent has presumably attached itself to the 1,4-positions in the conjugated system.

There are two possible explanations that may account for the types of products found, *i.e.*, ring-chain tautomerism* and *cis-trans* isomerism. At first sight the former might seem the more probable. The principal method hitherto successfully applied to determine the amount of cyclic form present in the ketonic acids, namely, titration with various strengths of barium hydroxide (37, 38, 39, 41, 42), could not be used with the compounds described in this paper, because of their insolubility. Also, if this were a case of mobile tautomerism it would have to be assumed that the Grignard reagent reacted more rapidly than any tautomeric change could take place. However, the results found fell very clearly into two classes, which would not be expected of such a variety of tautomeric substances. It would account for the varied values found in the two exceptions cited.

* *See references Nos. 17, 21, 34, 35, 37, 38, 39, 41, 42, 44 and 45.*

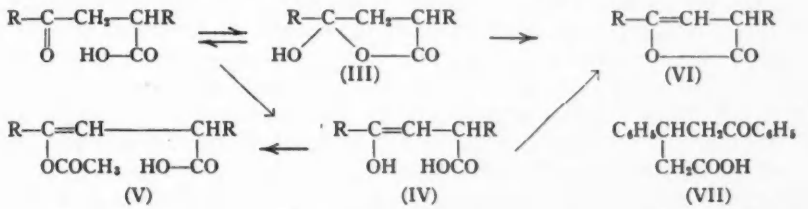
The alternative possibility is that of *cis-trans* isomerism, the acids having a *cis* structure giving rise to esters of both the open and the cyclic forms, which would obviously be impossible with a substance having a *trans* configuration. This would be in agreement with the observation that the open-chain acids never gave a single product derived from the cyclic form. The most conclusive proof would be the isolation of three esters, corresponding to the cyclic, *cis*, and *trans* forms, as accomplished by Lutz in the case of certain aroyl crotonic acids (24–28). It was shown in the first paper (3) that the lactols gave cyclic and open-chain esters, but in no case has it been possible to isolate the third. Geometrical isomers are often intraconvertible by means of exposure to sunlight under various conditions, and the *trans* modification is often colored; in this work substances of each class (I, II) were unchanged after six weeks' exposure and all were colorless. This may be inconclusive, since such properties are not necessarily universal.

It would seem unusual to find that a substituent group in one ring was a determining factor in giving rise to a *cis* or *trans* structure, especially since both types of products were found indiscriminately. However, Lutz has found that groups in rings do have a very marked effect in determining the configuration of diaroylethylenes, so not only is this objection without weight, but the parallel affords a strong argument that the differences are really of a stereochemical nature. Further, reduction of the 4-chloro homologue, known to be cyclic, gives a saturated acid which has an open-chain configuration, according to the Grignard results. Since a *cis* configuration forces the groups closer together (the reverse is true of open-chain saturated types, as Lutz found in analogous series), this evidence also favors a stereochemical explanation. It seems probable, therefore, that this is an additional example of *cis-trans* isomerism, the *cis* acids having a cyclic structure, which is impossible in the case of those having a *trans* configuration.

Since tertiary alcohols that have no hydrogen in a position available for dehydration form chlorides when treated with acetyl chloride, representatives of each class were treated with this reagent. It was found to form chlorides with those (II) that the Grignard reagent had shown to be cyclic (with one exception) but to be without action on those (I) having the open-chain form, thus acting as a confirmatory agent. The reaction with acetyl chloride is not a reliable criterion for distinguishing the enolic form in ordinary tautomerism, but these cases are not exactly comparable; moreover, if the substance has a cyclic structure, the product is a chloride, whereas an enol has never been known to form a chloride with this reagent.

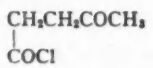
Two simpler saturated series were then investigated. The $\alpha$-aryl-$\beta$-aroylpropionic acids were already available, since they had been made for use in the first part of the investigation, and the $\beta$-aroylpropionic acids were easily prepared. There was no evidence of a cyclic form of the latter, but in a few instances, the former gave, in the Grignard machine (Table I), reproducible intermediate values that could be interpreted as evidence for the existence of a mixture of the two possible forms (there is no opportunity for stereo- or geometrical isomerism). Two are apparently entirely cyclic.

In these two series, the hydroxyl group of any lactol (III) could be eliminated as water, so that dehydration would be anticipated with acetyl chloride and it would not be expected to be of value as a confirmatory agent. An enolic form (IV) is also a possibility that cannot be disregarded; it could not only yield an ester (V) but it could form an unsaturated lactone (VI) by loss of water with the carboxyl group.

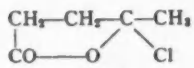



Varied results were obtained in both series. The prolonged action of acetyl chloride on the $\alpha$-aryl-$\beta$-aroylpropionic acids brought about some kind of dimerization, the nature of which was not determined because of the intractability of the products. Neither the nature of the substituent groups nor the length of the chain seemed to have any effect, because the same types of products were obtained from the $\delta$-ketonic acid (VII) as from the mesitoyl* $\gamma$-acid. Since addition reactions of the carbonyl group in a mesitoyl $\gamma$-acid are excluded, the dimerization, if it involves the group at all, must be through an enolic modification. There was one exception—the mesitoyl homologue gave an unsaturated lactone in addition to the substance of high melting point.

An unexpected result was obtained when the acetyl chloride solutions of these acids (including VII) were cautiously treated with methyl alcohol—methyl esters were formed. Easy esterification may be a property of ketonic acids, since Cowley and Schuette (10) have found that unless precautions were observed, alcoholic solutions of levulinic acid gave the 2,4-dinitrophenylhydrazone of the ester during the short time required to carry out the reaction. The production of esters may thus be accounted for, the hydrogen chloride formed from the acetyl chloride acting as a catalyst. An alternative possibility is that of interchange of radicals, even though the few recorded cases of interchange between an acid and an acid chloride took several hours (34, 36, 48), whereas the longest time involved in this series was 30 min. To explain the ease of formation of the esters, it would have to be assumed that (i) the acid chlorides are formed first, by a rapid interchange of radicals, and (ii) the acid chlorides have the open-chain structure (VIII). The only anomaly would then be Bredt's cyclic chloride (IX) of levulinic acid (9), which is discussed below.




(VIII)




(IX)

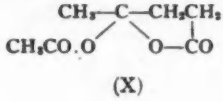
**Mesitoyl* = $-COC_6H_2(CH_3)_3$-2,4,6.

In the simplest series, the β-aroylpropionic acids, except the mesitoyl, gave unsaturated lactones; the mesitoyl gave an enolic acetate (V).

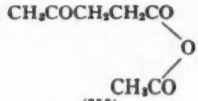
Since the products in the three series are not alike, the mechanism of the action of acetyl chloride must be different in the three. In the first series, in which it reacts only with those shown to be lactols by the Grignard reagent, the mechanism is similar to that of triphenylcarbinol, whatever that may be. In the other series it would be possible to account for the results by assuming that the acetyl chloride was first added to the carbonyl group (33); such an addition product could readily give rise to either enolic acetates or unsaturated lactones, both of which were found. Such an assumption is excluded because the mesityl compounds gave the same types of products, and no addition reactions of the carbonyl group of a mesitylenic ketone are known (13, 14, 20). This assumption was excluded long ago in the levulinic acid series by Bredt (9) who found that the esters did not react with acetyl chloride. Further, as previously mentioned, the halide did not react with the keto acids in the acrylic acid series described in this paper, which were shown by means of the Grignard machine to have an open-chain configuration. The remaining possibility is that of direct reaction with an enolic form; this seems plausible and accounts satisfactorily for the various types of products. The enol may form an ester directly or an unsaturated lactone by loss of water with the carboxyl group.

Lees (23) found that aliphatic ketones formed enol benzoates with benzoyl chloride but did not react with acetyl chloride. In this work it has been found that cyclohexanone reacts with acetyl chloride to form the known enol acetate (29). All efforts to prepare an enolic acetate or benzoate of acetomesitylene were unsuccessful, although it is known that this ketone is enolized by the Grignard reagent (20).

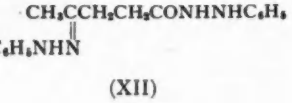
When levulinic acid is treated with acetyl chloride, it forms a chloride to which Bredt (9) finally assigned the cyclic structure (IX), because on treatment with silver acetate it gave "acetyl levulinic acid". The principal argument for a cyclic structure (X) for the latter was the production of the angelica lactones from it by loss of acetic acid, and the reverse—addition of acetic acid to the lactones to give the "acetate". The evidence by no means conclusively excludes an open-chain mixed anhydride structure (XI)—in fact, this structure seems the more probable one in view of the other known reactions and some of the facts presented above.



(X)



(XI)

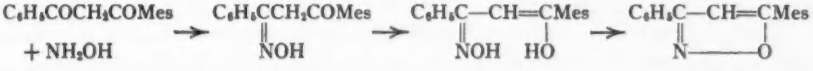


(XII)

Bredt found that all the supposed cyclic derivatives of levulinic acid, on treatment with phenylhydrazine, gave the phenylhydrazone of levulinic phenylhydrazide (XII); this involves no unusual assumptions if the open-chain formulas are used. Further, silver levulinate and acetyl chloride gave

the same "acetate", a procedure which is employed to form mixed anhydrides. Wolff (50) found that "levulinic acid chloride" in carbon disulphide was easily brominated, and that when the reaction mixture was treated in the usual manner, $\beta,\delta$-dibromolevulinic acid was produced. This is the ordinary bromination product of levulinic acid. The mechanism of formation of this bromination product is like that of any ketone having hydrogen in the position alpha to a carbonyl group.

It has long been known that lactones formed from enolized ketones are not regenerated when their solutions in alkalies are acidified, and that they are formed from the ketonic acids by the action of acetic anhydride, in exactly the same manner as ordinary ketones give enolic acetates; therefore, any evidence based on the lactones is inconclusive. As described above, the mesitylenic ketones, which cannot form addition products, give both an unsaturated lactone and an enol acetate, either of which could arise from an enolic form of the ketone, as outlined on page 385. In addition, benzoylmesitoylmethane readily gave an isoxazol; since mesityl ketones do not form oximes, the reaction undoubtedly proceeds as follows:




water being lost from an enolic modification.

In view of these facts, it seems extremely probable that lactone formation in the ketonic acids is brought about by the dehydration of an enolic form, and, therefore, in the case of levulinic acid the formation of the angelica lactones cannot be used as evidence for a cyclic form; whether the acetate is an intermediate product is uncertain. The available present evidence seems to be overwhelmingly in favor of open-chain structures for the levulinic acid series.

## Experimental

### *A. The Chalcones and Related Compounds*

All the required ketones were obtained by the usual methods. The yield of 2-chloro-5-methylacetophenone was increased from 34 to 85% by increasing the time of reaction to four hours (2). *p*-Carboxyacetophenone was obtained by heating *p*-bromoacetophenone with aqueous potassium cyanide in an autoclave, using a cuprous cyanide catalyst (12).

The unsaturated ketones* were prepared as previously described (3); the properties of the new compounds are given in Table II. The 4′-methyl homologue crystallized easily during the preparation if the temperature was kept below 30° C. (cf., Barat (6) ). The 4′-carboxy derivative was made by shaking a cold mixture of a 10% solution of sodium hydroxide and 50 cc. of water; in a few minutes the sodium salt of the desired acid separated and was used in this form in the next step. The yield was 97%. A sample was converted to the free acid, m.p. 232° C.** (43).

* *The benzalacetomesitylene was prepared by Mr. E. T. Henry, and the p-fluoro homologue by Mr. G. K. Dodd; their assistance is gratefully acknowledged.*

** *Melting points are corrected.*

TABLE II

PROPERTIES OF THE CHALCONES AND THE DIBROMIDES

| RCH=CHCOR′ | | | Dibromides | | | |
|---|---|---|---|---|---|---|
| R | R′ | B.p., °C. | M.p., °C. | Crystal form | Calcd., % | Found, % |
| $C_6H_5$ | 2-$ClC_6H_3CH_2$-5 | 195–200/6 mm. | 117 | Needles | Cl, 8.8<br>Br, 39.8 | 8.6<br>39.5 |
| $C_6H_5$ | 2-$CH_3C_6H_3$(i-$C_3H_7$)-5 | 205–210/12 mm. | 140–1 | Needles | Br, 37.7 | 37.5 |
| | | M.p., °C. | | | | |
| Piperonyl | $C_6H_4Cl$-4 | Needles, 128 | — | — | Cl, 12.4 | 12.2 |
| | | | 152 dec. | Plates | Cl, 8.0<br>Br, 35.8 | 7.9<br>35.5 |
| $C_6H_5$ | Mesityl | — | 131 | Rods | Br, 39.0 | 38.8 |
| $C_6H_5$ | $C_6H_4F$-4 | Plates, 76-77 | — | — | C, 79.8<br>H, 4.8 | 79.8<br>4.7 |

3,4-Methylenedioxybenzoyl-*p*-chlorobenzoylmethane (*A*) and benzoylmesitoylmethane (*B*) were prepared from the corresponding chalcone dibromides by the standard procedure* (1). α-Bromobenzal-2,4,6-trimethylacetophenone (*E*) was formed when the corresponding dibromide was treated with hydroxylamine by the procedure that has been shown to give isoxazols of known configuration (49), whereas 3-*p*-chlorobenzoyl-5-piperonylisoxazol (*C*) was obtained from the corresponding dibromide under the same conditions. The latter also resulted by the action of hydroxylamine on the diketone; 3-mesityl-5-phenylisoxazol (*D*) was obtained in a similar manner.

TABLE III

PROPERTIES OF THE DIKETONES, ISOXAZOLS, AND MONOBROMIDE

| Substance | Formula | M.p., °C. | Crystal form | Calcd., % | | Found, % | |
|---|---|---|---|---|---|---|---|
| | | | | C | H | C | H |
| *A*† | $C_{16}H_{11}O_4Cl$ | 151 | Leaflets | 63.5 | 3.6 | 63.4 | 4.0 |
| *B*‡ | $C_{18}H_{18}O_2$ | 84 | Plates | 81.2 | 6.7 | 81.1 | 7.0 |
| *C* | $C_{16}H_{10}O_3NCl$ | 180 | Leaflets | 64.0 | 3.3 | 63.8 | 3.8 |
| *D* | $C_{18}H_{17}ON$ | 76 | Plates | 82.1 | 6.4 | 82.3 | 6.8 |
| *E* | $C_{18}H_{17}OBr$ | 73 | Plates | 65.4 | 5.5 | 65.0 | 5.6 |

NOTE: † *Copper derivative, m.p. 290–292° C., dec.* ‡ *Copper derivative, m.p. 185° C., dec.*

*B. The Nitriles, Acids and Esters of the α-Aryl-β-aroyl propionic Acid Series*

These were also obtained by previously described methods; their properties are summarized in Tables IV, V and VI.

* *The authors are indebted to Mr. A. J. Cameron for the preparation of the substances in the piperonyl series, and to Mr. W. R. Carmichael for the mesityl compounds. Their co-operation is gratefully acknowledged.*

The preparation of the nitrile from the 4′-phenyl homologue required four hours' heating at the boiling point, using 20 cc. of alcohol and 5 cc. of chloroform per gram of unsaturated ketone; the 4′-carboxy derivative was obtained by using a mixture of the substances in the ratio of 3 moles of potassium cyanide and 1.5 moles of acetic acid to 1 mole of the sodium salt of the ketone, and stirring for two hours at 75° C. The clear solution was then acidified with acetic acid.

TABLE IV

PROPERTIES OF THE NITRILES, $RCH(CN)CH_2COR'$

| R | R′ | M.p., ° C. | Crystal form | Yield, % | Formula | Calcd., % | | Found, % |
|---|---|---|---|---|---|---|---|---|
| $C_6H_5$ | $C_6H_4F$–4 | 102 | Needles | 90 | $C_{16}H_{12}ONF$ | C 75.9 | | 75.6 |
| | | | | | | H 4.7 | | 4.6 |
| $C_6H_5$ | $C_6H_4C_6H_5$–4 | 176 | Prisms | 80 | $C_{22}H_{17}ON$ | N 4.8 | | 4.5 |
| $C_6H_5$ | $C_6H_4CH_3$–4 | 80 | Needles | 85 | $C_{17}H_{15}ON$ | N 5.6 | | 5.7 |
| $C_6H_5$ | $C_6H_4NO_2$–4 | 155 | Rods | 75 | $C_{16}H_{12}O_3N_2$ | N 10.0 | | 9.5, 9.7 |
| $C_6H_5$ | $C_6H_4COOH$–4 | 239 | Plates | 90 | $C_{17}H_{13}O_3N_2$ | N 5.0 | | 5.2 |
| $C_6H_5$ | 2–$ClC_6H_3CH_3$–5 | 76-77 | Needles | 85 | $C_{17}H_{14}ONCl$ | N 4.9 | | 5.1 |
| $C_6H_5$ | Mesityl | 77-78 | Needles | 80 | $C_{18}H_{19}ON$ | N 5.2 | | 5.4 |
| Piperonyl | $C_6H_4Cl$–4 | 129 | Leaflets | 80 | $C_{17}H_{12}O_2NCl$ | Cl 11.3 | | 11.1 |

TABLE V

PROPERTIES OF THE ACIDS, $RCH(COOH)CH_2COR'$

| R | R′ | M.p., ° C. | Crystal form | Yield, % | Formula | Calcd., % | | Found, % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | C | H |
| $C_6H_5$ | $C_6H_4F$–4 | 161 | Plates | 80 | $C_{16}H_{13}O_3F$ | 70.5 | 4.7 | 70.3 | 4.5 |
| $C_6H_5$ | $C_6H_4Br$–4 | 160 | Plates | 84 | $C_{16}H_{13}O_3Br$ | 57.6 | 3.9 | 57.8 | 4.0 |
| $C_6H_5$ | $C_6H_4CH_3$–4 | 152 | Leaflets | 90 | $C_{17}H_{16}O_3$ | 75.4 | 6.2 | 75.7 | 6.1 |
| $C_6H_5$ | Mesityl | 172 | Cubes | 50 | $C_{19}H_{20}O_3$ | 77.0 | 6.7 | 76.8 | 6.6 |
| Piperonyl | $C_6H_4Cl$–4 | 190 | Leaflets | 80 | $C_{17}H_{13}O_5Cl$ | 61.3 | 4.2 | 61.2 | 4.2 |

TABLE VI

PROPERTIES OF THE ESTERS, $RCH(COOCH_3)CH_2COR'$

| R | R′ | M.p., ° C. | Crystal form | Yield, % | Formula | Calcd., % | | Found, % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | C | H |
| $C_6H_5$* | $C_6H_4CH_3$–4 | 112 | Plates | 88 | $C_{18}H_{18}O_3$ | 76.6 | 6.4 | 77.0 | 5.9 |
| $C_6H_5$* | $C_6H_4C_6H_5$–4 | 157 | Needles | 70 | $C_{23}H_{20}O_3$ | 80.2 | 5.8 | 79.7 | 5.8 |
| $C_6H_5$† | $C_6H_3(CH_3)_2$–2,4,6 | 60-61 | Rods | 95 | $C_{20}H_{22}O_3$ | 77.4 | 7.1 | 77.3 | 7.1 |
| $C_6H_5$† | $C_6H_4F$–4 | 101 | Rods | 80 | $C_{17}H_{15}O_3F$ | 71.3 | 5.2 | 71.7 | 4.8 |
| $C_6H_5$* | $C_6H_4NO_2$–4 | 104 | Cubes | Very low | $C_{17}H_{15}O_5N$ | N | 4.4 | N | 4.0 |
| $C_6H_5$* | $C_6H_4COOCH_3$–4 | 110 | Plates | 80 | $C_{19}H_{18}O_5$ | 69.9 | 5.5 | 70.3 | 5.7 |
| $C_6H_5$† | 2–$ClC_6H_3CH_3$–5 | 80 | Rods | 94 | $C_{18}H_{17}O_3Cl$ | 68.3 | 5.5 | 68.4 | 5.7 |
| Piperonyl* | $C_6H_5$ | 121 | Rods | 66 | $C_{18}H_{16}O_5$ | 69.2 | 5.1 | 68.8 | 5.5 |
| Piperonyl* | $C_6H_4Cl$–4 | 109 | Rods | 72 | $C_{18}H_{15}O_5Cl$ | Cl | 9.9 | Cl | 10.0 |

* *Directly from nitrile;* † *through the acid.*

*C. Aroylpropionic Acids and Esters*

These were all prepared by the Friedel-Crafts reaction from succinic anhydride and the appropriate hydrocarbon (46). The three naphthalene derivatives were supplied through the kindness of Dr. L. F. Fieser, whose courtesy is gratefully acknowledged.

TABLE VII

PROPERTIES OF THE β-AROYLPROPIONIC ACIDS AND METHYL ESTER, $RCOCH_2CH_2COOH$

| Aryl group | M.p., ° C. | Crystal form | Formula | Calcd., % | | Found, % | |
|---|---|---|---|---|---|---|---|
| | | | | C | H | C | H |
| $C_6H_4Cl$–4 | 131 | Plates | $C_{10}H_9O_3Cl$ | 56.6 | 4.2 | 56.3 | 4.3 |
| Mesityl | 107 | Plates | $C_{13}H_{16}O_3 . 1/2H_2O$ | 70.9 | 7.7 | 70.8 | 7.4 |
| (Ester)$C_6H_4Cl$–4 | 63 | Leaflets | $C_{11}H_{11}O_3Cl$ | 58.4 | 4.5 | 58.8 | 4.5 |

*D. The Acrylic Acid Series*

The new lactols or ketonic acids, except the carboxy-substituted ones, were obtained essentially as previously described (3, 4). To obtain the ester, the components were refluxed for 10 min. only, using 2-gm. lots; the sodium salt which separated, was removed, and acidified with acetic acid. When it was dissolved in sodium hydroxide, or in the large amounts used in the preparation, the acid was obtained on acidification. It was not found possible to condense salicylic aldehyde with any of the γ-keto esters.

In general, the acids were insoluble in sodium bicarbonate. They all dissolved in dilute sodium hydroxide, but formed oils with a 10% solution; cumarin was found to act likewise. They gave brilliant colors with concentrated sulphuric acid—this was especially marked with those containing ether groups.

TABLE VIII

PROPERTIES OF THE LACTOLS OF KETONIC ACIDS*

| $R_1$ | $R_2$ | $R_3$ | Crystal form | M.p., ° C. | Formula | Calcd., % | | Found, % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | C | H | C | H |
| $C_6H_5$ | $C_6H_5$ | Mesityl | Plates | 250, d. | $C_{26}H_{24}O_2$ | 81.2 | 6.2 | 81.1 | 6.4 |
| Piperonyl | $C_6H_5$ | $C_6H_4Cl$–4 | Needles | 153 | $C_{24}H_{15}O_3Cl$ | 68.8 | 3.7 | 69.0 | 3.8 |
| Piperonyl | $C_6H_5$ | $C_6H_5$ | Rods | 138 | $C_{24}H_{18}O_5$ | 74.6 | 4.7 | 74.4 | 5.0 |
| $C_6H_5$ | $C_6H_5$ | $C_6H_4C_6H_5$–4 | Needles | 144 | $C_{29}H_{20}O_2$ | 83.2 | 5.3 | 82.9 | 5.3 |
| $C_6H_5$ | $C_6H_5$ | $C_6H_4CH_3$–4 | Prisms | 133 | $C_{24}H_{20}O_2$ | 80.9 | 5.6 | 80.6 | 5.8 |
| $C_6H_5$ | $C_6H_5$ | $C_6H_4COOH$–4 | Needles | 240 | $C_{24}H_{18}O_5$ | 74.6 | 4.7 | 74.8 | 4.4 |
| $C_6H_5$ | $C_6H_5$ | $C_6H_4COOCH_3$–4 | Plates | 137 | $C_{25}H_{20}O_5$ | 75.0 | 5.0 | 75.3 | 5.2 |
| $C_6H_5$ | $C_6H_4Cl$–2 | $C_6H_4Cl$–4 | Needles | 147 | $C_{28}H_{16}O_2Cl_2$ | Cl | 17.3 | Cl | 16.9 |
| Anisyl | $C_6H_4OCH_3$–2 | $C_6H_5$ | Needles | 126 | $C_{25}H_{22}O_5$ | — | — | — | — |
| $C_6H_5$ | $C_6H_4Cl$–2 | $C_6H_5$ | Rods | 98 | $C_{24}H_{17}O_3Cl$ | Cl | 9.4 | Cl | 9.5 |
| Anisyl | $C_6H_4Cl$–2 | $C_6H_5$ | Needles | 154 | $C_{24}H_{19}O_4Cl$ | Cl | 8.7 | Cl | 8.7 |
| Anisyl | α-Furyl | $C_6H_5$ | Needles | 121 | $C_{28}H_{18}O_5$ | 72.9 | 5.0 | 72.4 | 4.9 |

*$R_2CH_2$
$R_1C=C-COR_3$
COOH

As stated in the introduction, the method of indirect titration was of no value. The substances were insoluble in any excess of $N/100$ barium hydroxide, hot or cold; if enough alcohol was added to yield a clear solution, all gave values corresponding to 100% free acid. In the hope of obtaining results of value, all the substances were treated under the same conditions; the sample was dissolved in 3 cc. of methyl alcohol, 25 cc. of base added, and the excess found by back-titration. In most cases, the addition of the alkali solution caused the formation of a precipitate, so the values obtained are probably more a measure of solubility relation than anything else. The figures ranged from 45 to 95% of acid, open-chain form; the actual results were considered inconclusive, and so are omitted here. Under the same conditions, benzoic acid showed 100%, and cumarin 40%, acid.

*E. Action of the Grignard Reagent*

The results obtained are summarized in Table I. The values are considered accurate to 10%, except where ether groups are present.

In the case of all the β-aroylpropionic acids there was one active hydrogen and one addition, so the acids are not listed separately. The aroyl groups were benzoyl, *p*-methyl- and *p*-chlorobenzoyl, mesitoyl, 2-acenaphthoyl, 6-methylnaphthoyl-2, and 2,6-dimethylnaphthoyl-1. All formed sparingly soluble magnesium complexes, which made it necessary to prolong the treatment for several hours. An apparent anomaly was encountered with the mesitoyl compound; the active hydrogen was 1.5, 1.6, 1.6, with 0.9 addition in each case. This agrees with the values found on combustion (Table VII), where the acid is indicated as containing water of crystallization.

*F. Action of Acetyl Chloride*

*a. Chlorides in the acrylic acid series.* In addition to the tertiary chlorides described in the first paper (3), a homologue was obtained from the *p*-carboxy lactol; m.p., 197° C. Calcd. for $C_{24}H_{17}O_4Cl$: Cl, 8.7%. Found: Cl, 8.7%.

The acids that were indicated as having an open-chain configuration by the Grignard machine were unchanged by acetyl chloride.* The acid chloride of the 4′-phenyl homologue was obtained by the use of phosphorus pentachloride. On refluxing a benzene solution of 1 gm. of the latter with 2 gm. of the keto acid for an hour, removing the liquids *in vacuo* while heating on the steam bath, and recrystallizing from ether-petroleum ether, the chloride was obtained, m.p. 150° C. Calcd. for $C_{29}H_{21}O_2Cl$; Cl, 8.1%. Found: Cl, 7.9%. It formed a *p*-bromoanilide (4), m.p. 182° C.

Since tertiary chlorides like trityl chloride form substituted anilides with aromatic amines (11, 16), the formation of a *p*-bromoanilide is of no use for distinguishing the two series. Only one cyclic chloride, the one from the 4′-chloro homologue, was used, and it easily gave a substituted *p*-bromoaniline, m.p. 176° C. Calcd. for $C_{29}H_{21}O_2NClBr$: Hlg., 21.7%. Found: Hlg., 22.1%.

* *In this connection it may be noted that β-benzoylpropionic acid can be recrystallized unchanged from thionyl chloride and phosphorus trichloride.*

*b. The α-aryl-β-aroylpropionic acids.* When acetyl chloride solutions of these acids were allowed to evaporate spontaneously, fine crystalline powders were left. They were insoluble in the common solvents except dioxane, and insoluble in sodium hydroxide. The product from α-phenyl-β-(*p*-chlorobenzoyl) propionic acid, m.p. 235° C. with decomposition, showed two active hydrogens and one addition in the Grignard machine. Calcd. for $C_{23}H_{24}O_5Cl_2$: C, 68.7; H, 4.3; Cl, 12.7%; mol. wt., 559. Found: C, 68.8, 69.0; H, 4.7, 4.3; Cl, 12.8%; mol. wt., 525. The other homologues have the following melting points (all with decomposition): phenyl, 295° C.; *p*-fluorophenyl, 247° C.; *p*-methylphenyl, 270° C.

When the procedure was altered by destroying the excess of solvent by cautious addition of methyl alcohol, after a small amount of the high melting solid had separated, the solution deposited the methyl ester of the acid on evaporation. Thus, the ester from the simplest member, α-phenyl-β-benzoylpropionic acid, melted at 102° C.; it was at first thought to be the known α,γ-diphenyl-crotolactone, m.p. 103° C. (5), but a mixed melting point was 85–90° C. It did not decolorize bromine and proved to be identical with an authentic specimen of the ester.

There was but one exception in the series—the mesityl homologue gave a crotolactone; it formed needles, m.p. 126° C., decolorized bromine instantly, and dissolved in sodium hydroxide solution; on acidification α-phenyl-β-mesitoylpropionic acid was obtained. The lactone is soluble in the common solvents. Calcd. for $C_{19}H_{17}O_2$: C, 82.3; H, 6.1%. Found: C, 82.0; H, 6.5%.

β-Phenyl-γ-benzoylbutyric acid. This δ-ketonic acid also gave an ester when treated as indicated above. A trace of a solid of high melting point was formed by the action of acetyl chloride alone, but most of the acid was recovered unchanged. Barat (6) stated that he obtained a lactone by such a procedure, but he gave no experimental details, and his statement was not confirmed.

α-Phenyl-β-benzyl-β-(4-chlorobenzoyl) propionic acid was formed by the reduction of the corresponding acrylic acid with zinc dust and 80% acetic acid. It formed rods, m.p. 173–174° C., soluble in ether, alcohol, and benzene. Calcd. for $C_{23}H_{19}O_3Cl$: C, 73.0; H, 5.0%. Found: C, 72.6; H, 4.7%.

*c. The β-aroylpropionic acids.* (I) γ-(*p*-Chlorophenyl) crotolactone. A solution of 5 gm. of β-(*p*-chlorobenzoyl) propionic acid in 10 cc. of acetyl chloride was refluxed for two hours; on cooling, yellow needles separated. The color could not be completely removed by repeated recrystallization or by use of decolorizing carbon. It is soluble in the usual organic solvents, but insoluble in sodium carbonate solution. A bromide-bromate titration indicated 1.06 ethylenic linkages. Calcd. for $C_{10}H_8O_2Cl$: C, 61.5; H, 4.1%. Found: C, 61.2; H, 4.0%.

(II). Enol acetate of β-mesitoylpropionic acid. When β-mesitoylpropionic acid was treated in a similar manner, the solution became dark red, and purplish-red needles were deposited, m.p. 288–290° C. This substance may

be one of the "Pechmann dyes", some of which are known to be formed in an analogous manner (22). Calcd. for $C_{26}H_{24}O_4$: C, 78.0; H, 6.0%. Found: C, 78.1; H, 6.2%. The enol-acetate was obtained from the filtrate, and had a pink tinge which was never removed. It dissolved easily in sodium carbonate solution and was re-precipitated by acids. A bromide-bromate titration indicated 1.1 double bond. Calcd. for $C_{18}H_{18}O_4$: C, 68.6; H, 6.8; $C_2H_3O_2$, 22.5%. Found: C, 68.2; H, 6.6; $C_2H_3O_2$, 22.3%.

*d. Cyclohexanone, cyclohexenyl acetate.* A solution of 10 cc. of acetyl chloride and 10 gm. of cyclohexanone was refluxed for two hours and fractionally distilled; 5 gm. of a liquid, b.p. 175–178° C. at 763 mm. Hg. was collected. Since only the boiling point is given in the literature, a sample was prepared as directed by Mannich (29), and all properties compared; cyclohexyl acetate is also included in the table, for comparison.

TABLE IX

PROPERTIES OF CYCLOHEXANE DERIVATIVES

| Substance | B.p., ° C. 763 mm. | $D_{20}^{20}$ | $n_D^{20}$ | $M_D$ | |
|---|---|---|---|---|---|
| | | | | Calcd. | Found |
| Cyclohexenyl acetate | | | | | |
| By above method | 178 | 0.9726 | 1.4612 | 39.11 | 39.22 |
| By Mannich's | 178 | 0.9725 | 1.4610 | 39.11 | 39.20 |
| Cylcohexyl acetate | 174 | 0.9782 | 1.4440 | 38.59 | 38.56 |

*G. Acetomesitylene*

In an apparatus from which moisture was excluded, a mixture of 20 gm. each of acetomesitylene and *p*-chlorobenzoyl chloride was refluxed for 10 hr.; hydrogen chloride was slowly evolved. After washing with dilute sodium carbonate solution, which removed 1 gm. of *p*-chlorobenzoic acid, and fractionating the oil *in vacuo*, 11 gm. of ketone was recovered, and 7 gm. of *p*-chlorobenzoic anhydride was obtained. The residue was a pitch and there was no evidence of an ester. The small amount of water necessary for the reaction may have been fortuitously present or derived from the decomposition giving rise to the residue. From similar runs with benzoyl chloride, a complete separation of the mixture of ketone and benzoic anhydride that distilled together was almost impossible. However, there was no evidence of ester.

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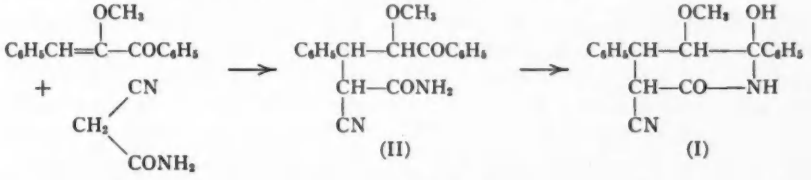
# THE ADDITION OF CYANOACETAMIDE TO α-METHOXY-BENZALACETOPHENONE[1]

BY C. F. H. ALLEN[2] AND J. A. SCARROW[3]

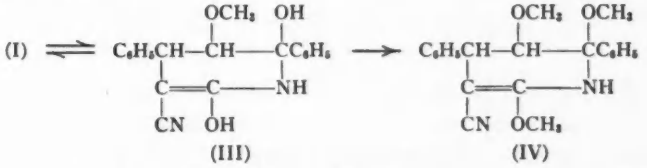

## Abstract

When attempts were made to add substances of the type HA to α-methoxybenzalacetophenone in the presence of an alkaline catalyst, it was found that the components were largely recovered unchanged, except in the case of cyanoacetamide; the addition product from the latter is a hydropyridine. The hydropyridine gives two distinct series of reaction products; in the first, the substances are all pyridine derivatives and always contain a methoxyl group. In the second, partial degradation has taken place, giving rise to cyclic derivatives of a δ-ketonic acid.


When cyanoacetamide was added to benzal α-methoxyacetophenone in the presence of a trace of sodium methylate, a hydropyridine (I) was obtained, instead of the isomeric open-chain δ-ketonic nitrile (II).



This addition product had no free carbonyl group, since it did not form an oxime, a semicarbazone, or a phenylhydrazone; it was soluble in sodium hydroxide and on methylation of the sodium salt with methyl sulphate gave the trimethylether (IV).

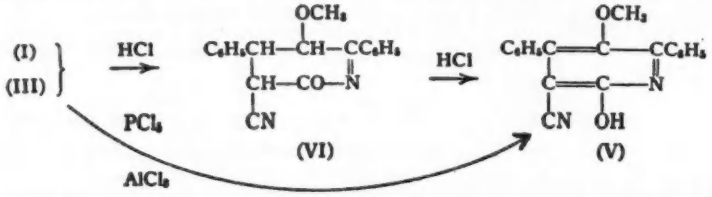


The formation of the ether would point to the tautomeric structure (III) for the addition product, but other reactions are interpreted more satisfactorily by the ketonic formula. In the following description of the substance and its reactions it will be noted that the methoxyl group was never eliminated (except on treatment with sulphuric acid, which led to an entirely different series of products) and consequently it was impossible to convert the pyridine derivative into known substances. The assumption that they are pyridine derivatives is based on their consistent reactions and properties, and by analogy with a homologous series (XI) with a hydrogen in place of

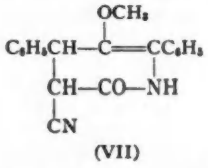
[1] *Manuscript received June 18, 1934.*
*Contribution from the Department of Chemistry, McGill University, Montreal, Canada. (This paper was read at the meeting of the Royal Society of Canada at Quebec, May, 1934.)*
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *Graduate Student and Demonstrator in Chemistry, McGill University.*

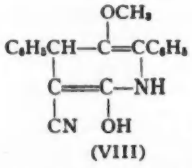
the methoxyl group (18). In all instances the reactions were strictly comparable; if unknown, they were determined in the course of this investigation.

The most characteristic reaction product was the highly substituted pyridine derivative (V); this substance was formed when phosphorus pentachloride or aluminium chloride was allowed to react with (I), but it appeared in nearly all reactions in varying amounts. Since the final ? step is a dehydrogenation, it is probable that the oxygen of the air removed the two

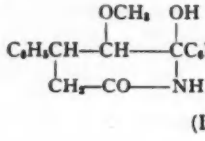


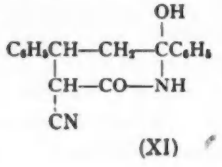

atoms of hydrogen during the prolonged time required for the usually slow reactions. For example, hydrogen chloride in chloroform first gave the pyridine (VI) by dehydration, but on standing several weeks (V) was obtained. Several alternative structures are possible for these substances; thus (VI) may be written as (VII) or (VIII). Since partially reduced pyridines are undoubtedly tautomeric substances, the formulas ascribed are a matter of individual preference.

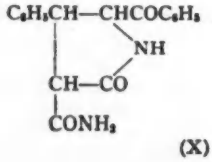


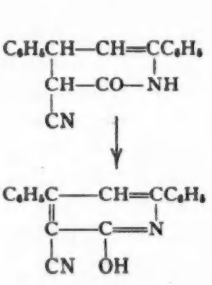




In addition to the hydroxypyridine (V), small amounts of (IX) and a lactam (X) were isolated from the mixture resulting from the action of anhydrous aluminium chloride. The homologous substance (XI), on similar treatment, gave the corresponding pyridine and tetrahydropyridine four times as rapidly.

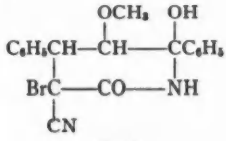


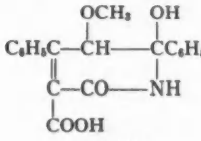



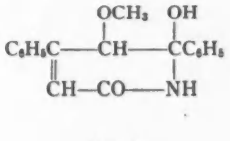
The action of bromine gave a complex mixture, from which were separated a bromo substitution product (XII), ammonium bromide, and three bromine-free products; two of the latter were readily identified as hydroxypyridine (V) and starting material (I). When the sodium salt of (I) was brominated, it gave a considerable quantity of (XII) and two bromine-free products; one of these was an acid (XIII), which lost carbon dioxide on warming to form the other (XIV), which was the third of the bromine-free products mentioned above. (XIII) is readily accounted for by hydrolysis and dehydrogenation; the amounts isolated were too small for further study.



(XII)



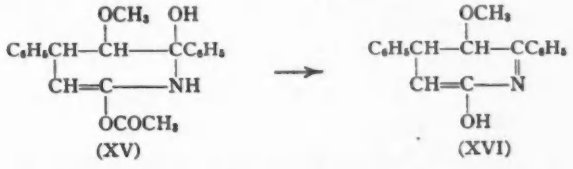
(XIII)



(XIV)

The bromine in (XII) was very unreactive; the substance dissolved easily in alcoholic alkali and was precipitated by acid practically unchanged, even after boiling with a $N/2$ solution of alcoholic potash for 2.5 hr. In boiling acetyl chloride it was slowly converted into the hydroxypyridine (V).

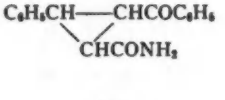
When attempts were made to dehydrate the addition product (I) with acetic anhydride in the presence of either sulphuric acid or pyridine, an acetate (XV) resulted, during the formation of which the nitrile group had disappeared. The acetyl residue was easily hydrolyzed with alcoholic sulphuric acid, but the process was accompanied by dehydration to yield (XVI). The structures assigned are subject to revision but seem to fit the available evidence better than any of the other possibilities. Kohler and Souther (18, pp. 2907-2908) described essentially similar results with (XI), and were able to detect the cyanide ion in one instance.



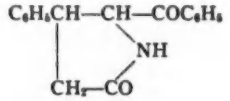
(XV) (XVI)

When the addition product was warmed in moderately concentrated sulphuric acid, a crystalline product and a gum were obtained on dilution. After exhausting all anticipated possibilities it was found that the solid formed a dinitrophenylhydrazone; therefore a free carbonyl group must have been present, and the six-membered ring structure of (I) must have been destroyed. As the cyano and methoxyl groups had also disappeared, the most probable structures were considered to be those of a cyclopropane

(XVII) or a lactam (XVIII). A stereoisomer, which gave the same dinitrophenylhydrazone resulted on treating an alcoholic solution of the substance with hydrogen chloride.



(XVII)



(XVIII)

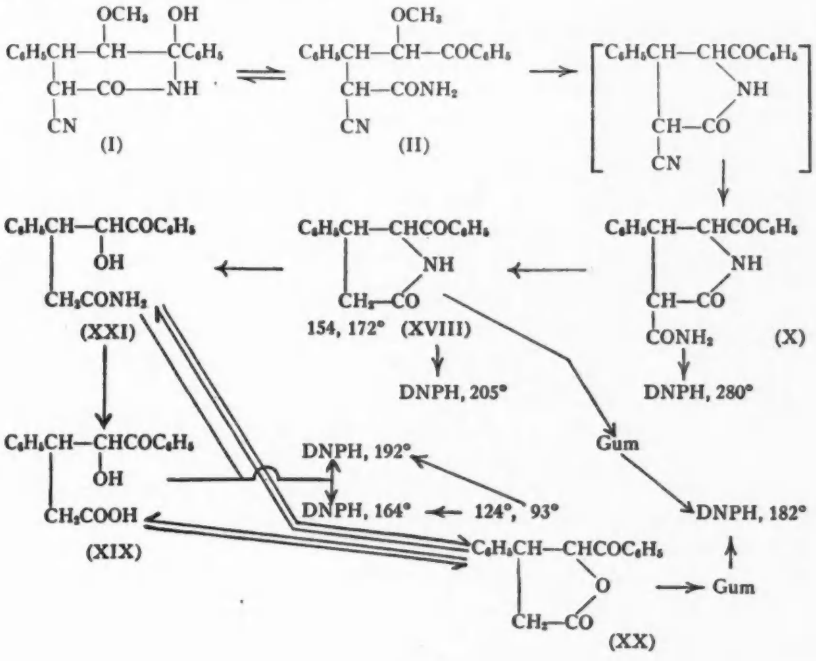
The substance was unaffected after boiling with phosphorus pentoxide in xylene, which made the cyclopropane-amide formula seem less probable. The solid dissolved very quickly when warmed with alkalies, ammonia being evolved, but the process was accompanied by complete decomposition. Since the nitrogen-free hydrolysis product of (XVIII) would be the hydroxy acid (XIX), which Kohler had already shown (14) to be very sensitive to both acids and bases, attention was turned to the gum; it gave a mixture of three dinitrophenylhydrazones. Two of these were isomeric and found to be identical with those prepared from the pair of stereoisomeric lactones (XX) or hydroxyacid (XIX), showing that the gum contained substances having the skeleton of a δ-ketonic acid. Some of the original gum was next carefully extracted with dilute alkali, and on acidification, the hydroxyacid and both lactones were separated.

When the lactones were treated with sulphuric acid in a similar manner, a gum resulted, from which a dinitrophenylhydrazone identical with the third one above was prepared. The analytical results indicated that it must be derived from some sort of a secondary product. On similar treatment, the lactam (XVIII) yielded a gum, that gave the same dinitrophenylhydrazone.

Attempts to synthesize the lactam by the action of ammonia on the lactone were unsuccessful. At ordinary temperatures a substance with almost the same melting point resulted, but the analyses indicated an addition product, possibly a hydroxyamide (XXI), which regenerated the lactone when treated with acetic anhydride. When treated with dinitrophenylhydrazone it gave the same dinitrophenylhydrazones as the lactones.

The course of the reaction from the addition product to the lactones may proceed in several ways. Since hydropyridines are always in equilibrium with open-chain compounds, the keto-amide (II) is undoubtedly the starting point. Whether hydrolysis of the cyano group or ring closure by loss of alcohol to form a cyano-lactam occurs first is uncertain. The latter possibility must be considered since a substance with properties corresponding to the amido-lactam (X) was isolated from the action of aluminium chloride

on (I). The series would then be (I) ⟶ (II) ⟶ cyano-lactam ⟶ (X) ⟶ carboxylactam ⟶ lactam (XVIII) ⟶ hydroxyamide (XXI) ⟶ lactone (XX) or hydroxyacid (XIX)




Cyanoacetamide was the only substance that it was found possible to add to α-methoxybenzalacetophenone by any procedure. The list of compounds used comprised benzyl and *p*-nitrobenzyl cyanides, desoxybenzoin, malonic, cyanoacetic, phenylacetic, and acetoacetic esters, nitromalonic aldehyde, anthrone, and cyclohexanone. A survey of the literature on this type of addition reactions indicates that cyanoacetamide adds most easily, judged by the time required and yield of product. *p*-Nitrobenzyl cyanide which has also been reported (1) to add readily, was recovered unchanged. Two suggestions for this lack of reaction may be considered. The first is the presence of a substituent in the alpha position, which is known to retard addition reactions to conjugated systems. Since it is only a slowing down of the rate it does not stop the reaction in the cases known, in which alpha substituents have been bromine (13, and experimental part of this paper), methyl (16), phenyl (9, 10, 11, 12), and carboalkoxy (5, 21), and the addends esters, ketones, nitriles, and aliphatic nitro compounds. The second is the

strongly positive nature of the methoxyl group. No quantitative data are available but Barat has stated that the more negative the ketonic end of the conjugated system, the more readily addition took place (3). In the above list of substituents methyl is the only (weakly, if at all) positive group, which would make the second suggestion seem more plausible. On the other hand, the real reason why the reaction takes place with cyanoacetamide only is probably because of the formation of the sparingly soluble hydropyridine. As this slowly separates it disturbs the equilibrium. Small amounts accumulate and the reaction proceeds in the direction of the cyclic compound. This explanation would also account for the ease with which Barat obtained hydroxypyridines in analogous instances.

## Experimental

*A. The Methoxy Ketones*

*ω-Methoxyacetophenone**, $CH_3OCH_2COC_6H_5$. The published directions are incomplete, in that sufficient details of the procedure, which has to be followed very carefully, are not given. To a solution of phenyl magnesium bromide (from 33 gm. of bromobenzene, 4.5 gm. of magnesium and 150 cc. of ether) was gradually added, with cooling, 10.4 gm. of methoxyacetonitrile† (23) in 10 cc. of ether). A brittle mass of the magnesium salt was formed, and this was pulverized with a heavy glass rod. After standing overnight, the mixture was added to 200 gm. of cracked ice and 20 cc. of concentrated sulphuric acid, the oily layer removed and the aqueous solution extracted with ether. The solvent was removed from the combined solutions, leaving a 70% yield of crude ketone, which was fractionated twice *in vacuo*. A fraction boiling at 100–130° C. at 15 mm. was obtained; yield, 40%. This fraction was then extracted with hot water (the two layers separated easily), which on chilling deposited large yellow rods. These could be used to inoculate an alcoholic solution of the oil obtained from the distillation. The purified ω-methoxyacetophenone had a melting point of 7.0–7.5° C.; b.p., 118–120° C. at 15 mm., 228–230° C. at 760 mm. The solubility in hot water is about 2.6 gm. per 100 cc. The semicarbazone, m.p. 129° C., and the 2,4-dinitrophenylhydrazone were prepared in the usual manner. The latter formed orange stubby rods, m.p. 191–192° C.; the analysis has been given elsewhere (24).

*α-Methoxybenzalacetophenone*. Though now relatively easily prepared (15) all this ketone used was obtained by the condensation of ω-methoxyacetophenone with benzaldehyde, essentially as described (20). The details were modified and improved somewhat, but will be omitted here, because of the superiority of the new method. When carefully purified it could be kept

* *The preliminary work and establishing of optimum conditions for securing this previously known (1,22) ketone by the Grignard reaction were done by Mr. A. M. Irvin at Tufts College, Mass., in 1928-1929, under the senior author's direction. The details of the final purification and results in the Grignard machine (17) were determined in the present work.*

† *A carefully purified sample gave off gas with methylmagnesium iodide; quantitative measurement in the Grignard machine (17) showed 50.5% addition and 5.9% active hydrogen; cf. Ref. 4, in which gas evolution with nitriles of low molecular weight has been noted previously.*

in a cool place unchanged for several years; m.p. 34–35° C. The 2,4-dinitrophenylhydrazone crystallized from alcohol in bright orange needles, m.p. 210–211° C. Calcd. for $C_{22}H_{18}O_5N_4$: N, 13.4%. Found: N, 13.3%.

*B. The Addition Reactions*

*2-Keto-3-cyano-5-methoxy-4,6-diphenyl-6-hydroxypiperidine, (I).* A mixture of sodium methylate (from 1.4 gm. of sodium and 70 cc. of dry methanol), 7.5 gm. of cyanoacetamide, and 12 gm. of α-methoxybenzalacetophenone was left at 40° C. for a week; crystals usually appeared after a day or could be induced to appear by scratching with a rod. These were filtered and combined with a second crop isolated from the acidified filtrate; the combined yield was 55–60%. After repeated recrystallization from methanol the substance formed large translucent prisms, m.p. 241–242° C. It is insoluble in cold alcohol, acetone, ethyl acetate and chloroform, moderately soluble in hot alcohol, and readily soluble in acetic acid and pyridine. Calcd. for $C_{19}H_{18}O_3N_2$: C, 70.8; H, 5.6; N, 8.7%. Found: C, 70.8; H, 5.6; N, 8.7%.* Careful examination of the residues and solutions revealed only unchanged components and traces of the hydroxypyridine (V).

The addition product was recovered unchanged from all attempts to secure an oxime, semicarbazone or phenylhydrazone. It dissolved in cold, dilute sodium hydroxide, and was precipitated unchanged on acidification.

The other addends, listed in the introduction, were used in very dry and ordinary alcohol, at the boiling point and room temperature, and in the presence of a trace or excess of sodium methylate, as well as diethylamine and piperidine. There was no evidence of an addition product in any instance.

*C. The Six-membered Series*

*3-Cyano-4,6-diphenyl-2,5,6-trimethoxytetrahydropyridine (IV).* This was very easily obtained by treating a solution of the addition product in an excess of 10% sodium hydroxide with an excess of methyl sulphate. The product slowly separated; it was filtered after an hour, washed and recrystallized from methanol, in which it is sparingly soluble. It melted at 290° C. with sublimation. Calcd. for $C_{21}H_{22}O_3N_2$: N, 8.0, $CH_3O$, 26.6%; mol. wt., 350. Found: N, 7.9; $CH_3O$, 27.0%; mol. wt., 344.

*2-Hydroxy-3-cyano-5-methoxy-4,6-diphenylpyridine (V).* The best yield was obtained by refluxing for two hours a mixture of 20 cc. of benzene, 3 gm. of phosphorus pentachloride, and an equal weight of the addition product. It was then well mixed with water; the benzene layer was drawn off and evaporated to dryness. The residue was triturated with cold, dilute sodium hydroxide to remove unchanged starting material, thoroughly washed and dried; the yield was 1.6 gm. (55%). On recrystallization from pyridine it

* *The nitrogen analyses were done by both semi-micro Dumas (19) and Kjeldahl methods. Since the latter was frequently low, the method was investigated, improved, and the results published (24). Almost without exception a low percentage of nitrogen was found in the cyclic nitrogen compounds by the original Kjeldahl method (2). The methoxyl groups were determined by a semi-micro Zeisel method (6). While good results were obtained with the partially reduced compounds, the pyridines and piperidines usually gave low values. These discrepancies often furnished a valuable clue as to the nature of the substance being analyzed.*

formed greenish-yellow rods, m.p. 318–320° C. with decomposition. It is soluble in pyridine, chloroform and acetic acid, but insoluble in the other common solvents. Calcd. for $C_{19}H_{14}O_2N_2$: N, 9.3; $CH_3O$, 10.2%. Found: N, 9.4; $CH_3O$, 9.5%. The hydroxypyridine was also obtained in the following ways: in the preparation of the addition product; from the latter by the action of aluminium chloride, hydrogen chloride in methanol or chloroform, alcoholic potash, and bromine in chloroform; from the gummy residue obtained by bromination, after boiling with alcoholic potash; from the dihydropyridine (VI) and hydrogen chloride in chloroform.

The hydroxypyridine dissolves in hot 2% sodium hydroxide solution and a sodium salt separates on cooling; acidification regenerates the original substance. The sodium salt was not alkylated by methyl sulphate. It was unaffected by phosphorus pentoxide in boiling xylene, but was destroyed by hydrogen iodide, giving indeterminate products.

*2-Keto-3-cyano-5-methoxy-4,6-diphenyltetrahydropyridine (VI).* Dry hydrogen chloride was passed into a suspension of 5 gm. of the addition product in cold, dry chloroform for four hours. The flask was closed and left for four days; the solution was then washed free from acid by water, and the chloroform layer evaporated. The residue was separated with difficulty into the tetrahydro compound and the hydroxypyridine (V). It dissolved very slowly in dilute sodium hydroxide. The yield was 2 gm. (40%); it is insoluble in benzene, ether, and cold methanol, but soluble in chloroform, acetic acid, pyridine, and hot methanol, from which it separates in prisms, m.p. 233–235° C. Calcd. for $C_{19}H_{16}O_2N_2$: N, 9.2; $OCH_3$, 10.2%. Found: N, 8.8; $CH_3O$, 10.2%. A chloroform solution, saturated with hydrogen chloride and allowed to stand for four weeks, was partially transformed into the hydroxypyridine.

*2-Keto-5-methoxy-4,6-diphenyl-6-hydroxypiperidine (IX).* A benzene solution of 20 gm. of aluminium chloride and 10 gm. of addition product was refluxed for seven hours, poured into water, made strongly alkaline, and warmed to 40° C. The insoluble solid was filtered and extracted with methanol. On cooling, the piperidine derivative separated as needles, which, after purification, had a melting point of 155° C.; yield, 5%. Calcd. for $C_{18}H_{19}O_3N$: N, 4.7; $CH_3O$, 10.4%. Found: N, 4.6; $CH_3O$, 10.0%. The hydroxypyridine (V) was isolated from the alkaline solution by appropriate manipulations.

By a similar treatment of (XI), the corresponding tetrahydro- and hydroxypyridines were isolated from the large number of substances formed.

*Action of Bromine. (a) In acetic acid.* To a boiling mixture of 5 gm. of the addition product, 50 cc. of acetic acid, and a drop of acetone, was slowly added an acetic acid solution of bromine at such a rate as to maintain a deep red color in the mixture—this required four hours and used up two equivalents of bromine. On cooling, some ammonium bromide separated. The solution was poured into water and left for 15 hr., during which time a mass of pliable, greenish filaments, up to 2 in. in length, separated on the surface. These

were removed and dried; it was impossible to purify them, although they have been kept for two years apparently unchanged. The melting point was 125–130° C. with decomposition. Found: N, 6.3; Br, 14.7; $CH_3O$, 3.1%. When placed in methanol they crumbled and a brownish sediment was deposited; the solution left a brownish gum. The sediment was tediously separated into starting material, hydroxypyridine (V), the bromo compound (XII) (60% yield) and the bromine-free (XIV) (2–5% yield).

2-Keto-3-bromo-3-cyano-5-methoxy-4,6-diphenyl-6-hydroxytetrahydropyridine (XII), forms pale yellow needles, m.p. 292–293° C., insoluble in the Skelly-Solves, ether, alcohol, and benzene, but soluble in acetic acid and chloroform. Calcd. for $C_{19}H_{17}O_3N_2Br$: N, 7.0; Br, 19.9; $CH_3O$, 7.7%. Found: N, 7.2; Br, 19.5; $CH_3O$, 7.4%. It was unaffected by acetic anhydride (at 40° C.), phosphorus pentoxide in boiling xylene, and sulphuric acid; it gave some of the hydroxypyridine (V) when refluxed for three hours with acetyl chloride. It dissolved in dilute sodium hydroxide and was reprecipitated by mineral acids; it was recovered nearly quantitatively after 2.5 hr. refluxing with $N/2$ alcoholic potash.

2-Keto-5-methoxy-4,6-diphenyl-6-hydroxytetrahydropyridine (XIV), is insoluble in alkali and crystallizes from alcohol in tiny prisms, m.p. 252–253° C. Calcd. for $C_{18}H_{17}O_3N$: N, 4.7; $CH_3O$, 10.5%. Found: N, 4.6; $CH_3O$, 10.0%.

*(b) In alkaline solution.* To the sodium methylate prepared from 0.3 gm. of sodium and 100 cc. of methanol was added 4.2 gm. of the addition product, and at the boiling point, bromine added as fast as the violence of the action permitted (20 min.). After 24 hr. the solid that had separated was found to be unchanged material and the bromopiperidine (XII). On evaporation of the solution *in vacuo*, a new substance separated; after purification from methanol, it formed bright yellow needles, m.p. 149–150° C.; yield, 40%. Calcd. for $C_{18}H_{17}O_5N$: C, 67.4; H, 5.0; N, 4.1%. Found: C, 67.9; H, 4.7; N, 3.9%. 2-Keto-3-carboxy-5-methoxy-4,6-diphenyl-6-hydroxytetrahydropyridine dissolved readily in sodium bicarbonate solution and was reprecipitated by acids. When heated (180–200° C.) it lost carbon dioxide; on purification, the residue was the non-acidic (XIV).

*Action of Acetic Anhydride. 2-Acetyl-5-methoxy-4,6-diphenyl-6-hydroxytetrahydropyridine (XV).* A mixture of 2 gm. of the addition product, 125 cc. of acetic anhydride (smaller amounts led to very poor yields) and two drops of concentrated sulphuric acid (or 10 cc. of pyridine), was refluxed for two hours and worked up in the usual manner, decolorizing with Darco during the methanol recrystallization. It formed needles, very soluble in hot methanol (but almost insoluble at 10° C.), m.p. 126° C. Calcd. for $C_{20}H_{21}O_4N$: N, 4.1; $CH_3O$, 9.2; $CH_3CO$, 13.3%. Found: N, 4.4; $CH_3O$, 8.9; $CH_3CO$, 13.5%. On hydrolysis, and from the residual solution after determining the acetyl content by Perkin's method (7), 2-hydroxy-5-methoxy-4,6-diphenyldihydropyridine (XVI) was quantitatively obtained. It separated in cubes from the alcohols, m.p. 173–174° C. Calcd. for $C_{18}H_{17}O_2N$: N, 5.0; $CH_3O$, 11.1%. Found: N, 5.0; $CH_3O$, 10.8%.

*D. The Five-membered Series*

*4-Phenyl-5-benzoylpyrrolidone-2 (XVIII).* A mixture of 20 cc. of concentrated sulphuric acid and 10 cc. of water was cooled to 50–60° C., 1 gm. of the addition product added, and the mixture warmed on the steam bath for 3.5 hr. A precipitate was obtained on pouring the solution into water; this was dissolved in methanol and separated in long needles, m.p. 172° C. An isomeric form resulted on attempted esterification of the product by the use of alcoholic hydrogen chloride; it was found essential to keep the temperature low, otherwise only oils resulted. The solvent was removed in a vacuum desiccator, and needles, m.p. 153–154° C., slowly separated. Calcd. for $C_{17}H_{14}O_2N$: C, 77.0; H, 5.7; N, 5.3%. Found: (172° C.) C, 77.5; H, 5.5; N, 5.3%; (153° C.) N, 5.1%. Both gave the same 2,4-dinitrophenylhydrazone; the latter separated from alcohol in yellow needles, m.p. 205° C. The lactam was unaffected by phosphorus pentoxide in xylene, very slowly destroyed by sulphuric acid (as below), but rapidly converted into black, untractable oils by alkalies with evolution of ammonia.

*3-Carbamyl-4-phenyl-5-benzoylpyrrolidone-2 (X).* This was isolated as a by-product from the reaction between aluminium chloride and the addition product, by fractional precipitation from the alkaline solution. It was freed from aluminium by a repetition of the process, followed by recrystallization from hot methanol; the yield was 5–10%. It is sparingly soluble in cold methanol and pyridine; it formed prisms, m.p. 273° C., with effervescence. Calcd. for $C_{18}H_{16}O_3N_2$: C, 70.1; H, 5.2; N, 9.1%. Found: C, 70.7; H, 5.2; N, 9.2%. The 2,4-dinitrophenylhydrazone crystallized from alcohol in brown prisms, m.p. 280–281° C.

The gum obtained by the original treatment with sulphuric acid was dissolved in chloroform and extracted with chilled, dilute sodium hydroxide. The alkaline solution was immediately placed in a separatory funnel containing cold ether and dilute hydrochloric acid; after shaking, and working up the ether layer, a solid resulted, which was separated with difficulty into a mixture of lactones (XX), and hydroxyacid (XIX). The lactones were converted into 2,4-dinitrophenylhydrazones which were more easily separated, and found to be identical with those formed from the lactones, synthesized by Kohler's method (14). The hydroxyacid and hydroxyamide (XXI), gave a mixture of the same dinitrophenylhydrazones. The lactone, m.p. 130° C., gave the derivative, bright yellow plates, m.p. 164° C., but the low melting lactone (98° C.) gave an isomer; orange-yellow plates, m.p. 192° C. Calcd. for $C_{22}H_{17}O_6N_4$: C, 62.0; H, 3.8%. Found: C, 62.4; H, 4.0%.

When the gum was directly treated with 2,4-dinitrophenylhydrazine, it gave a mixture of three dinitrophenylhydrazones, two of which were those described above; the third formed needles from alcohol, m.p. 182° C. Found: N, 11.2, 11.0%; mol. wt., 510. This same dinitrophenylhydrazone was also formed from gum resulting from treatment of the lactones or lactam with sulphuric acid under the same conditions as were used with the addition product.

*β-Phenyl-γ-benzoyl-γ-hydroxybutyramide (?) (XXI).* A solution of the lactone in dry methanol was saturated with ammonia and allowed to stand at room temperature for several days. On evaporation, prisms, m.p. 173° C. (mixed melting point with (XVIII), 155–160° C.) resulted. Calcd. for $C_{17}H_{16}O_3N$: C, 72.4; H, 5.7%. Found: C, 72.5; H, 5.5%. When heated in a sealed tube, a small amount of (XXI) was formed, along with much black oil.

*E. p-Nitrobenzyl Cyanide and α-Brombenzalacetophenone; Formation of a Mixture of 1-Cyano-1-p-nitrophenyl-2-benzoyl-3-phenylcyclopropanes*

To a warm mixture of 5.7 gm. of the bromoketone, 3.2 gm. of the nitrile and 25 cc. of dry methanol was added, drop by drop, a solution of sodium methylate, until the color, at first green, and then magenta, had changed to a pure blue (the characteristic color given by the cyclopropane and alkalies). It was acidified with a few drops of acetic acid, and on scratching, the solid separated; yield, 2.5 gm. (48%). It was separated into a small amount of unused nitrile, and the two stereoisomeric cyclopropanes, m.p. 144° and 151° C. respectively. The residue was a green gum.

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## REVIEWS AND NOTES

### Laboratory Test of Ultrasonic Depth Sounder

Recently the National Research Laboratories were asked to test a Huson Ultrasonic Depth Sounder, of the type commonly installed on launches, and having a range of about 200 ft. Since it is believed this is the first time a depth sounder has been directly tested for such depths in a laboratory, it was thought worthwhile to record the results obtained.

The Huson sounder is made up of two identical magnetostriction rod oscillators, one used as transmitter, the other as receiver. The power used for driving the transmitter is derived from a motor-generator, operating at 400 volts. This voltage is used to charge a condenser, which discharges at periodic intervals through the coil wound around the rod oscillator. A damped oscillation is thus emitted into the water. The second rod picks up the echo, vibrates slightly, and hence induces a minute electric current in its surrounding coil. This current is amplified and eventually passes into a metal contact, moving rapidly up and down on a piece of paper, which rolls slowly past the contact. The paper has been made conducting by a potassium iodide solution, and as the current passes through it a discoloration occurs. The position of the drawn-out spot thus marked is determined by the distance the contact has travelled between the time of the transmission of the signal and the time of its reception, and hence is a measure of the distance the signal has travelled, *i.e.*, the depth of the sea-bottom. The spots on the paper occur close together and result in a line which is really a profile of the ocean floor over which the boat is moving.

To carry out the test, the basin used for testing models of aeroplane floats and ships' hulls was employed. This basin is 396 ft. long, 6 ft. deep and 9 ft. wide. Additional equipment in connection with the tank includes a light, metal carriage which can be moved up and down the length of the basin as required. On this carriage was mounted the gear used for sounding, the ultrasonic transmitter and receiver being clamped on a piece of angle iron, one above the other, so that they were immersed in the water, pointing horizontally, one about a foot above and the other about a foot below the mid-depth point. This was arranged so that one end of the tank would act as the reflector, the equivalent of various depths being obtained by moving the carriage to different distances from the end.

In an actual installation, the ultrasonic transmitter and receiver are placed inside the ship's hull, so that the energy must go and come through $\frac{1}{8}$ in. or more of metal. To simulate this condition, a large sheet of duralumin, $\frac{1}{8}$ in. thick, was hung from the carriage, in front of the transmitter and receiver.

The frequency of this sounder is only 16,000 cycles, so that a conical horn is used in connection with the rod oscillator to concentrate the energy into a beam. The formula for the half-angle of the beam is $\sin\frac{1}{2}\varphi = 1.2\ \lambda/D$,

where $\lambda$ is the wave-length of the sound in the liquid (water), and D is the diameter of the transmitter. Since the aperture of the cone is only six inches, the above formula shows that the total angle of the beam is over 90°. With such a wide angle, it was feared that multiple reflections from the sides of the tank would cause a very indefinite echo, and that poorly defined depth measurements would most probably occur.

In Fig. 1 is shown a photograph of one of the records obtained. The ordinates represent depths; the abscissas, time intervals, or, in general, horizontal distances, the distances depending on the speed of the boat. Since this particular record was obtained as indicated above, the ordinates really represent distances from the end, or ends, of the tank. The abscissas, similarly, represent only time intervals.

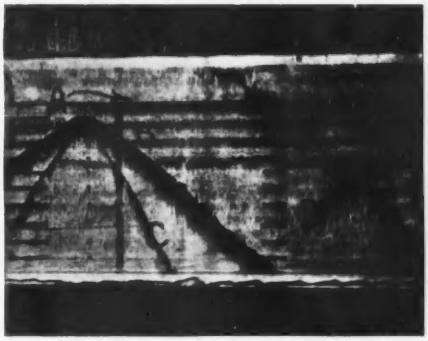

FIG. 1. *Photograph of record obtained with depth sounder.*

In the figure, the line marked "A" is the "zero" line. In an actual installation, it would indicate the surface of the water.

B is the line of depth, and was obtained by moving the carriage at a constant speed, first, towards one end, and later, away from it. Where B is parallel to the zero line, the carriage was not moving. As the "depth" increased, it will be noticed that the line became somewhat irregular. This presumably was caused by reflections from the tank walls.

Line C is the double depth line, caused by the sound being reflected from the end of the tank, then from the metal in front of the transmitter, again from the end of the tank, until finally the beam reached the receiver. This line often appears in practical sounding, on account of reflections from the surface of the water.

Line D was caused by the sound being reflected from the metal directly in front of the transmitter to the opposite end of the tank, back to the metal, and into the receiver. This line has a slope opposite to lines B and C, because the end of the tank from which its reflections occurred was approached (this "depth" became less) as the carriage receded from the main reflecting end.

Line E was caused by reflection from the first end, plus an additional reflection from each end. The beam of sound responsible for it had a path length of twice the distance from the transmitter to the reflecting end of the tank, plus twice the total length of the basin. Hence line E represents depths of 400 to 600 ft.

The results here recorded have shown that, in spite of some previous misgivings, the testing of a depth sounder in a narrow tank is quite feasible,

even up to equivalent depths of 400 ft. Some slight irregularities in the depth line were apparently caused by reflections from the walls of the basin, but aside from that, such reflections were of negligible effect.

In conclusion the writer would like to thank Mr. K. F. Tupper, in charge of the water basin, and Captain C. B. Potts for assistance rendered in connection with this test.

DIVISION OF PHYSICS AND ENGINEERING,
NATIONAL RESEARCH LABORATORIES,
OTTAWA, CANADA.

GEO. S. FIELD.

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